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STUDIES
ON
SMOKING AND HEALTH
1979-1983
RETROSPECT AND PROSPECT

December 1985

The Council for the Study of Smoking and Health

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The Council for the Study of Smoking and Health was founded in 1973 to administer fairly the many studies on smoking and health entrusted by the Japan Tobacco and Salt Public Corporation. The Council members consist of clinical and basic medical specialists.

The Council is a strictly impartial and independent organization which imparts a wide range of studies, allots funds, and evaluates the results.

There have been 116 different research projects over the past twelve years (1973-1984) with a total of 1,685 million yen allotted by the Council for these studies.

The reports of these studies have been reported by the researchers to the medical and scientific meetings, and have been published in scientific journals.

Following the reformation of the Japan Tobacco and Salt Public Corporation to Japan Tobacco Inc. on April 1, 1985, the Council was relocated to the Tobacco Research Institute (TASC) at the following address.

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STUDIES
ON
SMOKING AND HEALTH
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More than twenty-five years have elapsed since the Japan Tobacco and Salt Public Corporation began to conduct research projects on smoking and health in 1957.

Summarizing its work during the previous years, the Council for the Study of Smoking and Health presented the first report in 1979 entitled " Studies on Smoking and Health, 1957-1978, Retrospect and Prospect " (written in Japanese).

Five full years have followed the presentation of this first report. The Council thus presents herewith a second report which summarizes the results obtained from 1979 to 1983, and elucidates further prospects, so that this study project on smoking and health may more effectively be carried out.

The Council for the Study of Smoking and Health

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CONTENTS

I. Smoking and Cancer

[1] Basic Scientific Aspects 1

[2] Clinical Aspects 21

II. Smoking and the Cardiovascular System 40

III. Smoking and the Respiratory System 58

IV. Smoking and Physiology and Pharmacology 72

[1] Basic Scientific Aspects 72

[2] Behavior 84

V. Smoking Effects on Pregnant Women or Fetuses, and on Endocrine System and Metabolism 95

[1] Pregnant Women and Fetuses 95

[2] Endocrinology and Metabolism.....102

VI. Passive Smoking 108

VVI. Psychiatry 117

Conclusion 125

List of Investigators 126

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I. SMOKING AND CANCER

[1] BASIC SCIENTIFIC ASPECTS

1. Significance of studies

The presence of epidemiological data which implicate a close association between smoking and carcinogenesis in humans necessitates urgently basic scientific studies which elucidate which component(s) of tobacco is responsible for the transformation of cells and by what mechanism this takes place. At the same time, the results of such study should lead to the ways for the prevention of cancer.

In this study, basic research was conducted on the following topics: the influence of smoking on the occurrence of lesions such as tumors; the development of quick testing methods for mutagens and carcinogens and their application; the relationship between tobacco components and the drug-metabolizing enzyme system; and the influences on gene expression.

2. Progress and main results of studies

1) Influences of smoking; morphological and pathological studies

(1) Particle size of tobacco smoke and deposition in the airway region

Tobacco smoke is an aggregate of particulate matter; thus the particle size is the most important factor in

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considering the deposition of tobacco smoke in the airway. Kawai et al. felt that there was a discrepancy between the main region of lung tumor development (i.e., the central portion) and the theoretically expected deposition of tobacco smoke in the airway region, in view of the fact that the particle size of tobacco smoke is generally small. Therefore, they measured the diameter of the particles in tobacco smoke in a humidified environment close to free smoking conditions and found that the particles have a diameter of 0.8 to 1μm, two or three times larger than expected, thus indicating the possibility that tobacco smoke could easily be captured and deposited in the oral cavity and the upper airway.

(2) Influences of tobacco smoke on the respiratory system

Using a smoking apparatus for animal experiments, Ide et al. exposed mice to tobacco smoke for 6 hours a day for 18 months (daily exposure: equivalent to 20 "Hi-lite" cigarettes). In the treated mice, hypertrophy of Clara cells in the epithelium of the peripheral bronchus as well as hyperplasia of ciliary epithelial cells were noted. These changes, however, were reversible and did not lead to carcinogenesis. In an experiment by Shirasu et al., who exposed hamsters to tobacco smoke for 13 weeks (daily exposure equivalent to 30 cigarettes in two divided doses) the animals exhibited suppressed body

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weight gain, reduced efficiency of feeding, hyperplasia of the laryngeal mucosa and enhanced pulmonary macrophages. In addition, serum vitamin A level was decreased. These effects of tobacco smoke were suppressed by the administration of vitamin C, vitamin E, quercetin or disulfiram. Takemoto, who exposed newborn mice of the C57BL strain to tobacco smoke twice a day (6 days a week) starting within 24 hours after birth until the occurrence of death, found papilloma in the middle-size bronchi in 2 (1.9%) out of the 103 animals. However, it is generally difficult to induce tumor by exposing animals to tobacco smoke alone. Therefore, researchers at several laboratories studied the effects of smoking in combined treatment with known carcinogens on carcinogenesis. Among such studies, Takemoto reported a promotive action of smoking on the development of pulmonary tumors and pleural mesothelioma in asbestos-treated rats. Ide et al., who observed the occurrence of squamous cell carcinoma in "smoking" mice treated with a neblized N-methylnitrosourea (NMU), thought that NMU acts directly on the epithelium of the airway and that tobacco smoke promotes the creation of precancerous stages such as squamous metaplasia and dysplastic changes. Hayashi et al., who recognized the growth of epithelial cells around localized fibrous lesions in human

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lungs, studied the relationships between fibrous lesions and lung cancer in animals. They conceived that fibrous lesions, induced in the lungs of rats by intravenous injection of tetrachlorodifluoroethane, might become the region of deposition for carcinogens and lead to the growth of the surrounding epithelial cells. It was also suggested that the presence of such fibrous lesions might affect the physical motility of the lung, such as the respiratory air flow and the flow of blood and lymph, and lead to a reduction in the cleaning capacity of the lung.

For clinical studies on the process of histogenesis of lung cancer, Hanzawa et al. conducted a mass sputum cytology examination as well as follow-up studies on the cells showing of squamous metaplasia. In the examinations on a general population of 14,794 from 1979 to 1983, persons who had no abnormality accounted for 97.9% of the total. Those who were confirmed to have squamous metaplasia with mild or moderate nuclear atypia and needed a follow-up were accounted for 1.8%. Those who had the squamous metaplasia with marked atypia and immediately needed a careful examination accounted for 0.09%, and finally, those who were diagnosed as cancer were 4 people, (3 males and 1 female), accounting for 0.03%. Except for one female case, these other three patients were heavy smokers. The frequency of

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finding atypical cells in sputa tended to climb as the age and Brinkman Index (BI) increased. On the basis of autopsy data from 281 cases of lung cancer, Kitagawa et al. attested to the presence of close relationships between smoking and the genesis of squamous cell carcinoma, between smoking and squamous metaplasia and between squamous metaplasia and carcinogenesis. However, it remains an open question to what extent this model of progression (smoking → squamous metaplasia → squamous cell carcinoma) can apply.

In order to clinically study causal relationships between the habit of smoking and the development of lung cancer, Hanzawa et. al., further analyzed the association between the three factors: smoking, respiratory function and lung cancer. Subjects in this analysis consisted of 33 cases of lung cancer and 155 patients with non-respiratory diseases showing no clinically obvious abnormalities of the respiratory system. This analysis resulted in the following observations :

i) In the non-respiratory disease group, parameters of respiratory function were within the normal range in nonsmokers, but smokers showed a tendency to emphysema having disorders in the peripheral airway.

ii) In the lung cancer group, disorders in the central airway were found only in smokers, with increasing

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severity in heavy smokers. Disorders in the peripheral airway and a tendency to emphysema were found in both smokers and nonsmokers.

iii) Nonsmokers and mild smokers, in whom disorders of the peripheral airway was the main symptom, showed a high incidence of adenocarcinoma or small cell carcinoma of peripheral origin, while in moderate and heavy smokers, who showed a marked tendency to disorders of the central airway, squamous cell carcinoma and large cell carcinoma of central origin were the predominant cancer types.

iv) Female nonsmokers from the lung cancer group had more severe disorders in the peripheral airway than did nonsmokers from the non-respiratory disease group.

(3) Metastasis of tumors

By morphological and functional analysis of blood vessels in tumor tissues, which are indispensable in supplying nutrition for growth of tumors, Satoh H.et al., tried to understand the role of such vessels in the proliferation of cancer cells. The analysis revealed that the blood vessels present in tumor tissues are newly formed ones made of passive blood vessel beds without self-control and that the blood flow therein

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is secondarily influenced by the host microcirculation. The speed of blood flow in tumors was much less than that of the microcirculation in normal tissues, and the vaso-pressure was also lower in tumor tissues; however, the hypertensive effect of angiotensin II caused a marked increase in the volume and speed of blood flow as well as in the area and pressure of blood vessels present in tumor tissues. When nicotine was given, its effects on the blood flow in tumor tissues differed greatly between individuals, although there was on the whole a tendency of reduction in the blood flow. Tanaka K. et al. studied the association of coagulation and fibrolysis (platelets, fibrin, etc.) with the formation of new blood vessels in tumors. They found through this study that the deposition of fibrin is closely related to the growth and metastasis of cancer cells. Hence, it is conceivable that deposition of fibrin or suppression of its decomposition inhibits growth and metastasis of cancer cells, directly or indirectly, through suppressing the formation of new blood vessels. Nicotine is known to enhance adhesion of platelets and to inhibit reversibly the fibrolytic potency of smooth muscle cells in the tunica media of blood vessels, so its effects on the formation of new blood vessels in tumors need to be further studied.

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(4) Carcinogenicity of tobacco constituents

Itoh N. et al. conducted a carcinogenicity study on carbazole, a component of tobacco smoke, in mice. When a diet containing 0.15 to 0.6 % carbazole was given to mice of the B6C3F1 strain for 96 weeks, a significant increase in hepatocellular carcinoma and hyperplastic nodules in the liver occurred, and development of papilloma and squamous cell carcinoma was also seen in the forestomach.

2) Development of testing methods for mutagens and carcinogens

Tobacco smoke contains substances which are suspected of being mutagenic or carcinogenic. It is of great importance to develop a method to detect such substances quickly and accurately. For reasons of time and cost, suspicious substances are subjected to preliminary screening using a mutagenicity test with microorganisms; suspicious substances are then verified with cultured cell systems. Thereafter compounds proven positive with these test are finally judged in terms of their carcinogenicity using animal experiments.

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(1) Test methods using microorganisms

Most mutagens and carcionogens exert their biological actions after being metabolized and activated. In the case of microorganisms, which have no metabolic enzyme system, the mutagenicity test requires the help of metabolic enzyme system present in mammals. Mutagenicity tests were performed by Matsushima et. al. on nitroso compounds that are usually present in tobacco smoke. These compounds were metabolised with the liver enzyme of hamsters or rats, and the mutagenicity of the resultant metabolites was compared. Using the parameter of the emergence of histidine-induced revertants of Salmonella strains TA 98 and TA 100, it was clear that hamsters have a much greater ability to unveil the mutagenicity of any of the structurally different nitroso compounds (dimethylnitrosamine, diethylnitrosamine, dibutylnitrosamine, nitrosopiperidine, etc.), and that the metabolic enzyme system of hamsters is more favorable than that of rats for mutagenicity testing of very small amounts of nitroso compounds. In addition, when variations in the mutagenicity of tar was studied for tobaccos with various sugar additives, the mutagenicity of tobacco tar was weakened with increasing contents of glucose, fructose, sucrose or sorbitol. In particular, when the sugar content of a cigarette was 0.3g or more, the mutagenicity was reduced

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by more than 60%. Since the sensitivity of the combination of Salmonella with the rat metabolic activation system was low not only for the detection of mutagenicity of nitroso compounds but for that of nitrofurans and hydrazines, it was pointed out in their report the usefulness of the R-plasmid-introduced WP 2 uvrA strain of E. coli for screening these compounds.

On the other hand, Minoda tried to obtain from culture filtrate and extracts of microorganisms factors that suppress and inactivate the mutagenic properties of tobacco smoke condensate. However, screening of about 160 strains of bacteria did not lead to the discovery of any bacterium having specific suppressive factors.

(2) Test methods using mammalian cell culture systems

Using short-term culture systems of human and rat tracheae, Takayama S. et al., developed a method for quantitative analysis of unscheduled DNA synthesis (UDS).
In their experiments, small sections of the trachea were cultured for several hours in the presence of a test substance and tritium-labelled thymidine to detect any incorporation of thymidine into cell DNA in non-S phases using autoradiography. Using rat tracheae, they screened about 50 substances. From these, UDS was induced by 7 direct-type

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carcinogenic substances and 13 substances requiring metabolism and activation for carcinogenic action. Each case was clearly in a dose-related fashion; induction of UDS was observed with most of the carcinogenic substances that can cause tumors in the respiratory system of animals. Non-carcinogenic substances did not induce UDS. Induction of UDS was also observed in human bronchi when treated with 4-nitroquinoline-1-oxide (4NQO), methylmethane sulfonate (MMS), methylnitrosourea, N-hydroxy-N-acethylaminofluorene or other substances. In addition, Takayama S.et al., detected a highly reproducible, overt dose-response relationship between 10 kinds of carconogenic substances and induction of UDS with the use of an organ culture system of human oral mucosa. A long-term culture system of rat tracheal epithelium was established by Kitamura et al., by treating the system with carcinogenic substances in the early stage of culture. With this system, they succeeded in culturing for an average duration of 70 weeks.

Using the parameter of drug-induced sister chromatid exchange (SCE) in peripheral lymphocytes, Oikawa et al., studied differences in sensitivity of the method between species and between individuals. The results of this study were as follows:

- Drug-induced SCE differs according to the species and the kind of agent. Namely, when treated with 4NQO,

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SCE is induced in humans at about twice the rate it would in rats, while MMS induces SCE equally in both species.

- In humans, the individual variance in SCE induction was small under certain conditions, and no sex-or age-related differences were noted. However, there were rare individuals who showed abnormally elevated SCE when treated with a specific agent.
- In cancer patients,the incidence of high sensitivity to MMS and Trp-P-2 was much higher than in healthy individuals. It is not known whether such a high incidence is the cause of carcinogenesis or the result of tumor-bearing.
- The habit of smoking elevated the incidence of non-inductive SCE but did not affect inductive SCE.

Carcinogenesis comprises two stages, i.e., initiation and promotion; so-called carcinogens have both of these effects. However, there are substances that have only one of these two effects and are called either initiators or promoters. Initiation is irreversible, while promotion is reversible. Tobacco is suspected of having a promotive effect, because epidemiological studies have shown that the incidence of lung canncer is gradually reduced to that of

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nonsmokers when smokers quit smoking. In this connection, Itoh Y. et al., tried to screeen promoters by using culture cell assay systems. The principle of their study was to quantitatively observe new synthesis of the EB virus premature antigen, which is induced by human lymphoblastoid cells (Raji cells) having the genome of EB virus. Although the mechanism of such induction has not yet been clarified, it has been found that some known promoters of skin cancer such as TPA, croton oil and teleocidin have the potency for such induction. Retionic acids, known as anti-promoters, inhibit such induction, so an experimental system with these substances is useful not only for the detection and identification of promoters but for the screening of anti-promoters. With this assay system, however, no substances showing a positive reaction were detected from various commercial tobacco products, tobacco tar, tobacco plants and soil sample from tobacco fields.

(3) Animal experiments

Takayama S.et al. made it possible to detect UDS in living organisms, although this was difficult in the past for technical reasons. The method devised by them involves pinching the back skin of a mouse with ring-shaped tongue forceps and injecting a carcinogenic substance and tritium-

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labelled thymidine. This is followed by observation of UDS using autoradiography. When treated with 4NQO, MMS, etc., silver particles of UDS were found on the nuclei of the epithelium and mesenchymal cells, showing an overt dose-response relationship. This technique is quantitatively excellent and highly sensitive, Also it seems to be a useful system for detecting the DNA-damaging property of tobacco components and additives in the skin.

In considering the relationship between smoking and carcinogenesis, the association with tumors in the urinary tract is being studied. In this connection, Itoh N. et al., screened substances that exert some effect on the stages of initiation or promotion of carcinogenesis in the urinary bladder. In an experiment involving carcinogenesis in the urinary bladder of rats, they treated animals with N-butyl-N-4-hydroxybutyl-nitrosamine as an initiator for 2 to 4 weeks, followed by administration of the test substances mixed with the diet for 24 to 36 weeks. In these rats, they histopathologically examined the urinary bladder and found precancerous lesions such as papillary or tuberous hyperplasia. Further, a study on the promoting effect of tobacco smoke constituents was made on carbazole and N-nitroso-pyrrolidine. No difference was noted between these two substances in terms of the occurrence of papilloma in the urinary bladder;

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however, carbazole caused a significant increase in tuberous hyperplasia. Further, the tobacco component quercetin was analyzed for both initiating and promoting effects, with negative results for both.

Other than the above-mentioned testing methods for mutagens and carcinogens, Sakanoue et al., established a method of quantitative analysis for the radioactive substance ^{210}Po and its nuclophilic type ^{210}Pb, which contaminate tobacco leaves through fall-out. When each part of the leaf of tobacco plants (Bright Yellow MC) and commercial cigarettes were examined with this method, the content of these substances was larger in the middle leaf than in the upper leaf; the content in the leaf was more than that in the leafstalk; and Japanese cigarettes contained a larger amount of these substances than did foreign ones. Although no association was noted with tar or nicotine, the tobacco leaves produced in Suzu City (Ishikawa Prefecture) contained a larger amount of these radioactive substances than those produced in Okazaki City (Aichi Pref.), reflecting the more frequent occurrence of fall-out in the districts facing the Sea of Japan than along the Pacific coast. In addition, the content of such substances in tobacco leaves grown outdoors was several tens of times larger than that in leaves raised

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in green house. When the distribution of ^{210}Po and ^{210}Pb was studied in smoking, ^{210}Pb remained in the ash, while ^{210}Po volatized and for the most part was found in the smoke.

3) Biochemical studies of tobacco constituents on living organisms

(1) Drug-metabolizing enzyme system

When an extraneous matter enters a living body, a metabolizing system acts to eliminate it from the body. Carcinogens and mutagens also follow a similar course, but may possibly be partially activated and interact cellular macromolecules, i.e. nucleic acids and proteins, causing carcinogenesis. A metabolic enzyme system with this kind of mechanism is the cytochrome P-450 system, which is chiefly localized in microsomes. Takayama S. et al. analyzed the metabolical activation of benzo(a)pyrene (BP) by the cytochrome P-450 system. It is known that diol epoxide, formed by the activation of BP, has DNA binding capacity, and is mutagenic as well as carcinogenic. On the basis of this knowledge, factors that might affect the binding reaction of diol epoxide with DNA were studied. Hence it was observed that deoxicating enzymes and some components such as serum, hume compounds and unsaturated

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fatty acid exert a considerable influence on such a reaction. The suppressive action of these substances seen in mutagenicity tests of BP may be explained by their inhibitory action against the binding of DNA with an active intermediate of BP, a diol epoxide.

Watanabe et al. studied drug metabolizing systems in the lung and liver of healthy humans and lung cancer patients. The activity of BP metabolizing eyzymes in the lung was found to be several times or several tens of times lower than that in the liver, and the level of cytochrome P-450 was also low. Tobacco smoke contains only a very small amount of BP, but it is noteworthy that the lung, which first encounters by BP, has a metabolic and activation system, although its potency is small. Watanabe et al. further studied the specificity of several kinds of cytochrome P-450 purified from the liver and lung of rats. Yamane et al. examined the effects of tar on the carcinogenic process in the lung; as well as on aluminum, which accumulates in human lungs in the course of years. Tar did not increase the number of mice with lung cancer following the administration of dimethylnitrosamine (DMN), but it gave rise to an increase in the number of tumor formations per mouse. They attributed this result to the tar-induced promotion

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of the metabolism and activation of DMN, which led to methylation of more DNAs in the lung than in other organs. Aluminum, on the other hand, significantly suppressed development of DMN-induced lung cancer and methylation of pulmonary DNA in mice. In addition, the effect of tar was opposite to that of aluminum on the binding reaction 1-nitropyrene to pulmonary DNA.

(2) Genes and their expression

It is known that the transcription plays a central role in the expression of genetic information.

Therefore, Endo et al., cloned specific class II genes, which

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have a variety of features. Clones obtained were cancer-specific clones, clones specific for inflammatory and shock reaction, and organ-specific clones. The next step would be, with the use of these clones, to study changes caused in the gene expression level by tobacco constituents. Hanaoka et al., on the other hand, using cultured cell systems, studied the role of poly ADP ribosylation, which has been considered associated with differentiation, transformation and repair of DNA damage in cells. In this study, they found that the reaction of poly ADP ribosylation, which modifies nucleoprotein, was not indispensable to the mitosis and proliferation of cells, but that suppression of this reaction led to induction of SCE which is unrelated to the UV radiation induced SCE. The action of the poly ADP ribosylating reaction in cells has not yet been fully clarified; we expect that studies on the influences of tobacco constituents will provide us with some clues to this issue.

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3. Directions for future studies

A large number of epidemiological reports have been published on the association between smoking and carcinogenesis. Smoking indeed brings about a variety of disorders in the respiratory system, and such lesions indicated that tobacco might be associated with carcinogenesis. However, it is quite difficult to induce cancer in animals by means of inhalation smoking. This difficulty may be attributed to differences between humans and animals in basic aspects, such as absorption, transport, metabolic enzyme systems and excretion. Large differences may also exist as extrinsic factors, for example, the conditions of exposure, the duration of exposure and the growth circumstance.

On the other hand, the carcinogenic potency of tar is very weak among a variety of known carcinogens. Although tobacco smoke or tar contains highly potent carcinogens like BP, their content is too small to explain the phenomenon of carcinogenesis. In terms of the effects of two simultaneously administered carcinogens, there are many reports indicating reciprocal inhibitory effects of the two factors, although there are also reports that suggest additive effects. Thus, there are many questions left open about the synergistic effects of multiple carcinogens and substances contained in tobacco.

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Along with the recent advancement in genetic engineering techniques, analysis at the DNA level has progressed, enabling identification of several cancer genes and revealing the fact that point mutation of DNA is closely related to carcinogenesis. In studying the association of smoking with cancer, it will be indispensable to undertake research at the gene level or the level of genetic expression. On the other hand, studies on the issues involving cancer cell-host relationships, such as the role of the host during growth of cancer cells and the characterization of metastatic cancer cells may understand the over-all views of the phenomenon of carcinogenesis. These issues having difficulties due to the involvement of many factors, however, should be solved to enable the prevention of cancer.

[2] CLINICAL ASPECTS

1. Significance of studies

Assuming that smoking causes lesions in humans, it would be natural to expect the lung to be a target organ of such action. Since Wynder made public the view attributing lung cancer to cigarettes at the end of 1950, a variety of studies have been conducted including epidemiological, clinicopathological and animal studies. These studies by nature require

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much time to obtain results of significance, but we believe that our commissioned research is steadily bearing fruit. In epidemiological studies, the application of advanced multifactor analysis has enabled preparation of a mortality distribution table for domestic districts, thus gradually clarifying differences between urban and rural areas or between northern and southern prefectures. Clinicopathologically, original cells of carcinogenesis in the bronchus have been indentified at the ultrastructural level, and this has led to the elucidation of extrinsic factors for carcinogenesis one after another. In terms of intrinsic factors, however, the study is still in the initial stage of clarifying the metabolism of carcinogens in experimental animal. It is also noteworthy that the issue of screening of early cancer cases and studies on the immune mechanism have made substantial progress over the last years.

2. Progress and main results of studies

1) Epidemiological studies

Epidemiological studies on the causal relationship of smoking to lung cancer have been given close attention. The genesis of lung cancer, however, involves many factors

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including smoking, and it is not known to what extent and by what mechanism smoking is associated with lung cancer. On this issue, Kurihara et al. have been gathering basic data for clarification. They computed age-adjusted death rates for each prefecture during 1973 and 1977 to be compared with the rates after 1958 (obtained for every fifth year) by the maximum likelihood method.

This study revealed that regional differences in mortality from lung cancer have become smaller in recent years when prefectures were compared, indicating that death from lung cancer is not necessarily shifting to industrial or rural areas. At present, however, it is difficult to determine the main cause of lung cancer. In numerical studies of the association of the age-adjusted death rate from lung cancer with tobacco consumption, temperature, humidity, rainfall, density of population, percentages of employees in primary, secondary and tertiary industries, number of automobiles and level of natural resources, no high coefficients of correlation were obtained.

The increase in lung cancer patients is currently of world-wide concern. Prevention would be the most desirable method to decrease the number of such patients. Meanwhile, over the past 30 years, diagnosis and treatment

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have greatly progressed enabling the 5-year survival rate of patients with early hilar-type lung cancer to near 100%. The same rate for early cancer in the lung is over 80%. Given this situation, epidemiological studies with analysis of death rates alone are not sufficient; the mortality index also needs to be studied in detail. A prerequisite for meeting this demand is to apply the cancer registration program done by the Ministry of Public Welfare to local stages of medicare as well. This is an urgent issue. We feel the necessity to advance this kind of study in collaboration with research groups engaged in the registration of cancer patients.

2) Pathological studies

Pathological studies aimed at clarifying the causes of human lung cancer are difficult; however, animal experiments and the preparation of experimental models are gradually contributing to such clarification. Kageyama et al. have been studying the original cancer cells of bronchiolar adenocarcinoma using material obtained from humans. Electron-microscopical observation of granules in adenocarcinoma cells, led to the classification of cells into the mucous cell type, type II pneumocyte type, Clara cell type, mixed type and non-granular type. In terms of cancer

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originating from type II alveolar cells, the relationship between inclusion bodies and smooth-surfaced endoplasmic reticulum has been clarified on the grounds that surfactant analogues produced from type II alveolar cells are found in the nucleus and that these surfactant analogues are micro-tubular inclusions that can be stained by anti-surfactants and that have strongly positive esterase reactions.

In the same field, Tauchi and Satoh T. have been studying the relationship between genesis, growth and progress of adenocarcinoma on the one hand and smoking on the other. Their study was focused on extrinsic and intrinsic factors of carcinogenesis, using the samples obtained with thoracotomy, whereby the removed lung was sliced serially and the largest section including lesions was subjected to study. This study was conducted in 260 males and 92 females. The incidence of adenocarcinoma of peripheral origin was conventionally thought to be high in females and to have no association with smoking; this study, however, revealed that the incidence in males increases with smoking, and that adenocarcinoma of hilar origin is also related to smoking. Thus, in the case of adenocarcinoma of peripheral origin, it became necessary to reconsider the association with smoking, and, at present, such a study is being made on each of this adenocarcinoma's sub-types. According to other studies,

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however, the association with smoking is rather poor when examined in smokers with lung cancer (Japanese females in Hawaii and aged females at Tokyo Metropolitan Yoikuin Hospital), although some variance was noted according to sub-type, region of genesis and progression. Particular attention is being paid to the association with age. An issue of importance to be clarified in the future is whether aging or smoking plays a more significant role in the development of such cancer.

In addition to these studies using clinical materials, Kanizawa et al. and Kimura Y. et al. are praised for having contributed greatly to the advancement of animal studies in this field. What deserves attention is the association of disturbed metabolism in Clara cells with carcinogenesis. In their study on the effect of isoproterenol, a beta-stimulator, to clarify the co-carcinogenic effect of 4NQO. These researchers found that Clara cells showed a similar effect with glycerol, a substance essential for metabolism of sugars and lipids. This result substantiated the fact that glycerol has both a promotive action and the effect of metabolizing and activating Clara cells in 4NQO-induced transformation, thus exerting a marked accelerative effect on carcinogenesis. The mechanism of such actions is being

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studied. Matsumura et al. is preparing an experimental model for the study of pulmonary adenocarcinoma in humans using adult dogs. This study, although still at the initial stage, aims at clarifying histogenesis. Namely, in this study, a disc made of 3,4-benzo(a) pyrene polymer was implanted under the pulmonary pleura of adult dogs receiving thoracotomy so that a follow-up study of carcinogenesis (the timing and progress) might be made with chest X-ray films, bronchoscopy and tumor markers. This method still has a few problems, including the technique of disc preparation, but it is a newly developed model utilizing a slow-release pellet and is expected to be applied for carcinogenic experiments of hilar squamous cell carcinoma.

In squamous cell carcinoma of hilar origin, both pathological and clinical studies have made rapid progress. Especially in clinical studies, the combination of bronchoscopy and cytological diagnosis of mainly atypical metaplastic cells has enabled the discovery and treatment of a large number of early hilar-type cancers, resulting in a 5-year survival rate approaching 100%. Now, objects of early diagnosis are being enlarged to highly atypical squamous metaplasia and intraepithelial carcinoma; basic research and experiments supporting this trend are making marked progress.

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Basic research, such as carcinogenic experiments in hamsters with a Hamburg II apparatus (Kobayashi), himan bronchus implantations and carcinogenesis experiments in nude mice (Yarita and Sawada) and a carcinogenic pellet implant experiment in fragments of the bronchus removed from adult dogs (Matsumura), have been experimentally developed by using a Krypton laser beam to localize carcinogenesis in dogs with squamous cell carcinoma (Hayata et al.). This technique is already in clinical use. Furthermore, a study with experimental dogs was conducted whereby 20-methyl-cholanthrene (20-MC) was repeatedly injected to the same site under the bronchial mucosa of adult dogs to induce squamous cell carcinoma. To such dogs, hematoporphyrin derivatives (HpD), highly oncotropic, were intravenously injected and then a Krypton laser beam was applied, resulting in positive fluorescent reaction in all cases of hilar-type cancer and weak fluorescence even in cases of highly atypical squamous metaplasia. This technique, which will be applied to an increasing number of human lung cancers, is quite effective. Further study is now being directed toward evaluating the extent of cancer, detecting highly atypical squamous metaplasia and establishing this technique as an objective one. In this connection, it also seems necessary to study the relationship between metaplastic lesions and

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the pathological picture of lesions upon biopsy and to question whether all regions with fluorescence indicate the presence of tumor-related lesions or not. It is also necessary to clarify the direction of forthcoming molecular-level studies on the mechanism of carcinogenesis in dogs.

Pathological studies incorporating analysis of carcinogenic factors were made by Mori et al. as well as by Tauchi and Satoh T.. Mori et al.classified the history of diseases (incl. smoking history) by way of coding various lesions in autopsied cases for the past 40 years, in an attempt to substantiate pathologically the epidemiologically shown causal relationship between lung cancer and smoking. Based on autopsied cases in this study, the incidence of lung cancer showed a remarkable increase. However, this tendency was seen in both smokers and non-smokers and in fact, the increase was comparatively higher in the non-smokers. In considering the cause of such an increase, they have indicated that factors such as improved diagnostic techniques and carcinogenic factors other than smoking also need to be considered.

Nagahama noticed that pneumoconiosis is often complicated by lung cancer, and he clinically studied the posibility of carcinogenesis when associated with smoking.

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When examined on the basis of four categories (pneumoconiosis, autopsied pneumoconiosis, lung cancer complicated pneumoconiosis and primary lung cancer), it was noted that pneumoconiosis has a high incidence in aged individuals and the prognosis has no association with smoking . Males with lung cancer and individuals with pneumoconiosis-complicated lung cancer were heavy smokers more frequently that were patients with pneumoconiosis, but there was no significant difference in tobacco consumption between these two lung cancer categories. They added that occupational inhalation of dust as seen in the case of pneumoconiosis has a higher risk of carcinogenesis of the lung than does smoking, but that smoking has a slightly higher risk for pneumoconiosis-complicated lung cancer.

It is said that squamous cell carcinoma is a type of lung cancer frequently seen in connection with smoking. There are, however, no nationwide statistics indicating the situation with regard to histological type or the incidence of each type. Thus, answers to these questions are sought so that an international comparison can be made. In reply to this demand, Katsuki and Sawada made a single-year survey in 1979 on 1304 lung cancer patients at 11 medical institutions and 900 patients with diseases other than lung cancer

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as controls. Data for individuals were entered into coding sheets and processed by computer. Cross-tabulations thus obtained were subjected to a test of significant differences on 30 parameters. This study was conducted on lung cancer cases occurring between 1973 and 1978. The percentage of symptom-bearing patients was 72.3% for both males and females. Symptoms observed were cough, sputum, and sputum cruentum (in the order of incidence). Cough and sputum were more often seen in lung cancers of central origin, and stethalgia has a high incidence in lung cancers of peripheral origin. The diagnosis of lung cancer was made on the basis of bronchoscopy in 67.6% of the total lung cancer cases, a percentage larger than expected. The positive reaction rate in sponge cytology was 79.6% and was large in lung cancer of central origin. In symptom-bearing subjects, epidermoid cancer was found in 74.7% and small cell carcinoma in 80.8%. In subjects with a history of smoking, epidermoid cancer had the highest incidence (44.7%); in subjects without a history of smoking, adenocarcinoma had the highest incidence (64.7%). In terms of the relationship between the age at which smoking began and the histological type of cancer, epidermoid cancer had a high percentage in each age group; this type of lung cancer had a high percentage also for each class of smoking duration. In total, the incidence of each histological type of lung

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cancer was 36.6% for epidermoid cancer, 36.1% for adeno-carcinoma, 11.1% for small cell carcinoma, 8.4% for large cell carcinoma, 5% for other types and 4.1% for unclassified cancers.

3) Mass screening

Over the past ten years or so, the number of persons receiving lung cancer screening and the cases of early cancer detected have shown a steady increase. Needless to say, such an increase is attributable to improvements in detection techniques such as presetting high risk groups (incl. smokers), improvements in evaluation of X-ray films and the widespread practice of cytology. Incorporating these points, Nakada et al. have studied and spplied more effective screening methods for many years, and detected and treated a larger number of early lung cancer cases than expected. Their method is to detect early cancer cases by periodic screening of the same group with wide coverage and to clarify the process of carcinogenesis by X-ray, cytologically and histologically, with a final objective of clarifying its relationship to smoking. Until now, indirect chest X-rays were taken of all inhabitants (about 120,000) from 28 local communities. Detailed examinations were

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conducted on 4.5%, and lung cancer was detected in 24 persons. What is characteristic of this method is that a file of records is available for tracing the time course of abnormal shadows appearing on X-ray films, contributing to reducing the necessity of additional detailed examination and to improving the accuracy of X-ray film evaluation. Of the 24 lung cancer cases, resection was conducted in 18 cases, of which 6 cases were established as early cancer. In sputum cytology, a new method combining Saccomanno's technique and the use of cysteine (a thrombolytic agent) was applied, whereby 18 lung cancer cases were detected from the 7500-odd subjects belonging to a high-risk group satisfying either of the following two conditions: (1) age over 50 and BI over 600 and (2) history of sputum cruentum within the latest three months. The total number of cases of lung cancer detected with the above technique, X-ray and cytology was 42, of which 34 cases (81%) received resection; 19 of the resected cases were established as early cancer. Of these 19 cases, 13 were squamous cell carcinoma which was detected with sputum cytology alone. Thus, both resection and early detection rates are increasing year after year.

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In addition to the study of Nakada et al., the screening program developed by Hayata et al. has attained successful results. In this program, mass screening with sputum cytology using a post-tube technique has been conducted on individuals of a high risk group selected from the employees of the Tokyo Metropolitan Government and general inhabitants. Subjects of this screening were about 4,200 individuals, of which 2 cases of highly atypical squamous metaplasia were detected and have been followed up. Recently, a TV campaign inviting TV watchers to submit to sputum cytology was made. Of the 2,000 individuals who participated in this screening, serious atypia was detected in 5 persons and lung cancer in 2 individuals, all of whom were smokers. One of the lung cancer cases was negative on chest X-ray films.

4) Host immunological competence

The high incidence of lung cancer or malignant tumors in other organs in heavy smokers may indicate reduced immunological competence,i.e., reduction in both humoral and cellmediated immune capacities. To document this possibility, Nishimoto et al. have been conducting an animal study since 1976. They intraperitoneally injected

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water-soluble components of tobacco tar and smoke to mice for analysis with various markers. In this study, they examined the influences of the substances on the T-cell forming capacity of spleen and thymus cells and the interleukin-2 producing capacity of spleen cells as well as the effects of thymosin fraction 5 on PHA and ConA reactions of spleen and thymus calls. Through these procedures, the picture of the immairing action of aqueous solutions of tobacco tar and tobacco smoke on the differentiation and maturity of T-cells began to be unveiled. Namely, both of these solutions suppress formation of T-Cells in spleen and thymus cells and production of interleukin-2 in spleen cells. Hoshino et al. have studied disturbances of the immune regulatory system in smokers since 1982.
They observed reduced activation of natural killer (NK) cells in smokers. Recently, their study has been enlarged to include the investigation on the interaction between autologous lung cancer cells and immune cells.
In this study, they measured autologous tumor cell killing (ATK) activity of lymphocytes in lung fluid in cases showing metastasis of lung cancer to the pleura, and made a comparison with the K-562 NK activity of the same lymphocytes.

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They are obtaining interesting results; for example, the immunological natural resistance of individuals against cancer cells could be measured more directly than by using the parameter of NK activity. In addition to a study on the immunocompetence of lung cancer patients with a history of smoking, in 1980 and 1981, Tanaka F. et al. studied a simple and quick immunological testing method using their own technique of leucocyte adherence inhibition (LAI) reaction employing a liquid extracted from lung tissues of fetuses. Between lung cancer patients with a history of smoking and those without such a history, there was no significant difference in the number of peripheral lymphocytes, the fraction of globulin, the fraction of immunoglobulin or the stimulation index (SI) in lymphocyte transformation. Similar results were also obtained in cases of other malignant tumors and benign lung diseases. Currently, diagnoses of lung cancer and its prognosis are being made on a trial basis with the microslide method, which is highly reproducible with the LAI test, indicating the possibility that evaluation of disease stage and the risk of recurrence can be made with the LAI reaction test. In an attempt to obtain a criterion for judging the indication for and prognosis of non specific immunotherapy after surgical treatment of lung cancer, Inoue et al. evaluated the effects of immunopotentiators.

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In this study, cultured human lung cancer cells were used as target cells and peripheral lymphocytes were separated from patients for calculation of the cellular disturbance rate. Clinical application of this technique has been tried for each disease stage, each histologic type and lymph nodes. The study has so far shown that the lymph nodes without metastasis have cellular impairing competence as is the case with peripheral blood, thus suggesting the presence of defense competence, although nonspecific. These researchers believe this technique to be useful in applying immunotherapy or improving its efficacy, but it will take more time before this technique can be used practically.

5) Others

Yamamoto et al., who studied histopathologically the interstitial reaction of the host lung tissues in lung cancer cases, classified the patterns of blood invasion into the following types: intra-intimal type, obstructive type and open type. They studied the association of these types with tissue types, and found that epidermoid cancer often shows the intra-intimal or obstructive type, while adenocarcinoma and small cell carcinoma often show the open

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type. These facts can affect the prognosis and results of therapy and can be helpful in determining the method of chemotherapy. These results were reported in 1979. The study was continued, and, in 1980, assessment of the possibility of resection in lung cancer patients as well as diagnosis of pulmonary embolism, pulmonary arteriovenous fistula, pulmonary emphysema and pulmonary hypertension were made with scientigrams of pulmonary circulation. These revealed that reduced blood flow in the diffuse lung is seen in heavy smokers and that this is attributable to increased resistance of blood vessels in the lung. This study is of significance in that the pulmonary scanning technique is clinically usefull in evaluating the effects of smoking, although little importance had previously been attached to this technique.

3. Directions for future studies

Clarification of the relationship between smoking and lung cancer is complicated and requires many different kinds of study. Epidemiologically, prospective studies that use morbidity rate, data obtained from nationwide cancer registers are playing a role in etiological studies on lung cancer, and this kind of study should show continued progress.

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Clinicopathological and animal carcinogenesis studies should complement each other; but clarification of the early stages of carcinogenesis will direct these studies to gene level studies such as those on de novo carcinogenesis. This kind of study will contribute especially to the clarification of the mechanism involved in cancers, such as pulmonary adenocarcinoma in females, which seemingly has no association with smoking. Further, mass screening will be conducted more widely than ever, and detection of early lung cancer in a larger number of females will contribute not only to early treatment of lung cancer but to clarification of the mechanism of carcinogenesis. Finally, progress in the technology of immune competence evaluation in hosts will clarify why some hosts are more susceptible to lung cancer than others.

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II. SMOKING AND THE CARDIOVASCULAR SYSYTEM

1. Significance of studies

Together with the westernization of the Japanese life style, cardiovascular diseases are increasing among Japanese people. Epidemiological studies conducted in Europe and America on many patients with cardiovascular diseases showed that risk factors for cardiovascular diseases are hypertension, hyperlipidemia, obesity and smoking. In Japan, Hirayama studied the relationship between smoking and circulatory diseases and found that smokers showed a significantly higher mortality from subarachnoid hemorrhage and ischemic heart diseases.

Nicotine and carbon monoxide have been discussed as factors responsible for the action of smoking on the cardiovascular system. Nicotine has long been known to cause elevated cardiac output, an increase in blood pressure and contraction of the peripheral blood vessels through indirect action on the sympathetic nerve-medullo-adrenal system or direct action on the motor center. In addition, it is possible that nicotine affects the metabolism of lipids and platelet adhesion-aggregation reaction, thus having a possible association with the development of atherosclerosis. There is also circumstantial evidence indicating that carbon monoxide affects the permeability of blood lipids through blood vessels.

In connection with the development of cardiovascular diseases and their harmful effect, it has been pointed out that not only the presence of angiosclerotic lesions but

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also functional factors (platelet functions, blood vessel spasm, etc.) may play significant roles. Therefore, it seems important to study the association of smoking with these factors.

2. Progress and main results of studies

1) Epidemiological studies

(1) Clinical epidemiological study on smoking and cardiovascular diseases (Yamori et al.)

This was an epidemiological study conducted in local communities to clarify the relationship between smoking on the one hand and hypertension and arteriosclerotic diseases on the other. First, the presence of a close association between the development of hypertension and excessive salt intake or deficiency in protein intake was found (1979). Next, the usefulness of the carboxyhemoglobin (HbCO) concentration was established as a quantitative objective parameter of cigarette consumption in evaluating the role of smoking (1980). With this parameter, a multiple regression analysis of risk factors was made, and a positive association of HbCO with the atherogenic index and a negative association of HbCO with HDL-cholesterol were found (1981). Further, it was revealed that smoking,

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obesity and alcohol can elevate hematocrit values and can thus become risk factors in atherosclerotic diseases like cerebral infarction (1982). Incidentally, in the framework of the "International Collaborative Work Program on Nutrition and Circulatory Diseases (Cardiac Study)" organized by WHO, these researchers detected a high correlation between daily urinary excretion of cotinine and cigarette consumption, thus documenting its suitability as a parameter of habitual cigarette consumption (1983).

Epidemiological surveys are important in clarifying the significance of smoking as a risk factor in atherosclerotic diseases. However, the significance of smoking as a risk factor varies among groups. For this reason, further studies will be needed to consider the involvement of genetic factors such as species, environment and alimentary conditions.

(2) Epidemiological study on chronic obstructive arterial diseases and smoking (Yamamoto et al.)

This was an epidemiological study on Buerger's disease, a recently increasing disease. The incidence of this disease in Toyama Prefecture is 4.78 per 100,000 population. Yamamoto et al. studied its clinical symptoms and treatment

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in detail (1981 and 1982). In terms of the association of this disease with smoking, this study could show only the possibility that smoking may worsen this disease based on data that 95% of the patients had a history of smoking 20 cigarettes a day. No causal relationship, however, could be established.

Because the size of the population in this study (several dozen) was too small, a larger population needs to be used in future studies.

2) Basic and experimental research

(1) Smoking and cardiovascular disease in animal models (Yamori et al.)

This study aimed at establishing experimental animal models and systems of experimentation for studying the relationship between smoking and hypertension or arteriosclerotic diseases. From the already established rat models capable of spontaneously developing hypertension and rats that easily develop cerebral apoplexy, they separated and established a strain of rats which develop myocardiopathy at a high incidence (1980) and another strain of rats which develop thrombosis at a high incidence (1982). Next, they established a technique for the separa-

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tion of plasma lipoprotein from rats for studying the metabolism of lipids in these animal models (1981). Furthermore, they succeeded in establishing a chronic smoking experimental system. Using this system, they studied the effects of smoking on the metabolism of lipids in the spontaneously hypertensive rats. This study revealed that smoking animals showed elevated hematocrit values, deterioration of hyperlipidemia and a higher atherogenic index compared to control animals, thus indicating that the influence of smoking can be largely modified by some genetic predisposition (1983).

This finding is interesting in comparison with the results of the epidemiological studies. The use of such model animals capable of spontaneous disease development will considerably advance studies on the association between smoking and hypertension or arteriosclerotic diseases and help to clarify the mechanism of such associations as well as advancing studies aimed at the prevention of such diseases.

(2) Hemodynamic effects of smoking on cardiovascular functions (Abe et al.)

This study aimed at clarifying the direct effects of tobacco constituents on coronary blood flow and the

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behavior of the left ventricle, using isolated perfused canine heart without being affected by the peripheral blood vessels or nervous-humoral factors. In this experimental system, a transient decrease in left ventricular pressure and transient bradycardia were noted after nicotine treatment, but these symptoms were followed by an elevation in cardiac contractility, thus indicating involvement of sympathetic and parasympathetic actions at the junction of nerves and muscles (1980). In addition, nicotine promoted ventricular relaxation; this action is the same as that seen when beta-receptors are stimulated, thus indicating the involvement of intrinsic catecholamines at the junction of nerves and muscles (1982). Furthermore, nicotine caused no hemodynamic change when it was administered to anesthetized dogs under thoracotomy with their alpha- and beta-sympathetic receptors blocked, while nicotine exerted an alpha-sympathetic action when administered to heart samples with the beta-receptors blocked. This finding suggests that nicotine's promotive action on cardiac contraction and relaxation is mediated by sympathetic receptors (1983).

As stated above, heart samples connected with a computerized volume servo-control system enable not only

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detailed hemodynamic analysis but also effective analysis of the direct effects of nicotine without being affected by nervous or humoral factors. In addition, the system allows the study of the involvement of intrinsic catecholamines. Thus, it will be quite useful in clarifying the mechanisms of the phenomena detected in clinical studies.

(3) Effects of tobacco smoke inhalation on the development of atherosclerosis (Asano et al.)

This study attempted to clarify the effects of tobacco smoke and the mechanisms of such effects through studying the influences of tobacco smoke on the development of experimental, diet-induced atherosclerosis. Rabbits from the group with high-cholesterol feeding and tobacco smoke inhalation showed, in contrast to rabbits from the non-inhalation group, elevated blood cholesterol levels, increased adhesion-aggregation reaction of leucocytes, crater-like degeneration of the aortic wall and occurrence of arteriolar twitch (1983).

The above results indicate the importance of the additive effects of risk factors in the development of atherosclerotic lesions. Therefore, clarification of

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the significance of the presence of nicotine and the mechanism of its action is awaited.

(4) Study of the rhinopharyngeal reflex and the cutaneous microcirculation at the time of inhalation of tobacco smoke (Asano et al.)

This study was aimed at elucidating the acute complex reactions which occur, via the rhinopharyngeal reflex, at the time of nasal inhalation of tobacco smoke. Change in the cutaneous microcirculation was used as a parameter. When rabbits were made to inhale tobacco smoke through the nose, the rhinopharyngeal reflex caused, suppression of respiratory activity, bradycardia, elevation in blood pressure as well as contraction of blood vessels in the cutaneous microcirculation. In addition, it was suggested that repeated inhalation can give rise to an adaptive phenomenon (1980 and 1981).

(5) Effects of smoking on the constitution and function of blood vessels (Toyoshima et al.)

Following detection of the transfer of nicotine to the retina in the mice (1979), this study was aimed at understanding the effects of nicotine on the formation of micro-vessels through studying how diabetic retinopathy occurs and worsens. This study, however, has achieved no

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successful results as yet (1980 through 1983). Needless to say, it takes much time to clarify the effect of smoking on the formation of blood vessels. Further efforts are needed for this clarification.

(6) Screening of antagonists to the effects of tobacco smoke components on the circulatory system (Suzuki et al.)

This study unveiled interesting pharmacological actions of bassianolide (extracted from pathogens of insects), which works as an antagonist to the pharmacological action of nicotine (1979 through 1982), but screening for the anti-nicotinic substances has not yet been conducted. This study, however, should contribute to future research because it has established a technique for detecting anti-nicotinic substances.

3) Clinical studies

(1) Influence of smoking on the blood pressure regulating system (Yoshimi et al.)

This study attempted to unveil the mechanisms of increased blood pressure and heart rate caused by smoking through studying the nervous and endocrine changes and the accompanying hemodynamic changes. When tobacco was

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smoked quickly, elevations in urine epinephrine and blood norepinephrine levels occurred, and these elevations were suppressed when alpha- and beta-sympathetic receptors were blocked, thus suggesting a close association of the nicotine-mediated sympathetic-medulloadrenal system with such changes (1979 through 1981). However, blood flow in the kidney was not greatly affected at the given level examined (1980). On the other hand, patients with essential hypertension showed an excessive reaction of the sympathetic-medulloadrenal system (1983), thus necessitating further study on the contribution of this system to the worsening of essential hypertension.

When the effects of smoking on daily life were studied through analysis of circadian changes in urine catecholamines, there was no difference between the value during smoking and that during non-smoking hours (1981). There are many difficulties in utilizing catecholamine levels as a parameter, because secretion of catecholamines differs greatly among individuals and can be changed by physical exercise. In addition, there are technical difficulties in measuring catecholamine levels. Thus, the establishment of methodology and experimental conditions are essential in conducting this kind of study.

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Furthermore, this study revealed increases in aldosterone and cortisol when subjects were loaded with smoking. In this connection, the study analyzed the ACTH-mediated mechanism (1979) and the effects of smoking on the secretion of anti-diuretic hormones and the metabolism of water and electrolytes (1982). However, no conclusion has yet been reached as to their mechanism or significance.

(2) Effects of smoking and nicotine on the autonomic nervous system and peripheral blood vessels (Imura et al.)

The effects of smoking and nicotine on the autonomic nervous system and peripheral blood vessels were studied by observing changes in blood catecholamine levels and blood flow by the Doppler method. This study enabled the demonstration of the relationship between the sympathetic nerve-mediated effect of nicotine on the one hand and elevation in blood epinephrine and norepinephrine levels and reduced blood flow in the peripheral arteries on the other. Marked reduction in peripheral blood flow was found in patients with Buerger's disease, suggesting that smoking is a factor responsible for its worsening (1979). However, blood flow in the brain was found to be increased in this study, indicating the necessity for further studies on this topic (1979).

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In addition, suppressed secretion of motilin (a gastrointestinal hormone) and pancreatic polypeptide during smoking were detected, thus indicating that smoking can affect the autonomic function of the digestive tract and can suppress the parasympathetic nervous system (1980). Although the effects of smoking on the cardiac branch of the vagus nerve (which belongs to the parasympathetic nervous system) and vaso-motor nerves were small (1982), this finding is not completely acceptable on the ground that many factors, i.e., the R-R intervals on ECG and the speed of aortic pulse wave, might have affected the parameters used in this study.

This study indicated that nicotine has sympathetic nerve-mediated influences on blood flow and functions of the digestive tract, and it raised questions to be solved, for example, the mechanism of nicotine's increasing cerebral blood flow and its selective action on the autonomic nervous system.

Furthermore, through comparative analysis on l-nicotine and d-nicotine, d-nicotine was found to be weak in promoting catecholamine secretion, increasing heart rate and reducing blood flow (1979 and 1980) and was also weak in its action on the autonomic functions of the digestive tract (1981),

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thus suggesting that d-nicotine has smaller pharmacological effects in humans as well.

(3) Physiological study of the effects of smoking on cardiovascular functions (Asano et al.)

The acute effects of smoking, under various conditions, were studied in healthy youths who smoked regularly. When alcohol was consumed, smoking exerted an additive effect in terms of elevating blood pressure and heart rate, whereas peripheral blood vessel contraction was offset (1979). When a physical load was performed, smoking exerted an additive effect on the cardiovascular system (1980 and 1982). The acute effects of smoking seen under these experimental conditions were also found in daily life when studied by a remote-control method (1981).

Also, cigarette smoking through a perforated filter was found to have a smaller effect on the blood flow (1982).

This study, which analyzed the effects of smoking on blood flow under various conditions, offered many suggestions on less hazardous smoking.

(4) Hemodynamic effects of smoking on the cardiovascular system (Abe et al.)

This study analyzed clinically the effects of smoking on the peripheral and left ventricular blood flow by means

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of a Doppler apparatus. In healthy subjects, smoking caused slight elevations in blood pressure and heart rate as well as in peripheral impedance, which represented the blood flow pattern in the common carotid and brachial arteries (1980). In terms of the effects of smoking on cardiac function, healthy individuals showed an elevation in the speed of left ventricular blood flow at the diastolic phase during smoking, thus suggesting promoted ventricular relaxation, which hints at the presence of a beta-stimulating action in smoking. This result was identical with those of the experiment using isolated heart samples removed from dogs receiving coronary perfusion. On the other hand, in patients with ischemic heart diseases, smoking caused reduction in the relaxation characteristic of the left ventricle, and no beta-stimulating action was observed in connection with smoking (1981 and 1983).

A technique to analyze the left ventricular relaxation by means of examining left ventricular blood inflow patterns by the pulse Doppler method was proposed by Abe et al. This technique seems to be highly useful in hemodynamic analysis during smoking. Now, elucidation of the mechanism involved in reduction in left ventricular relaxation seen in ischemic disease patients is needed. Further,

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it seems that the relationship between smoking and heart diseases can be clarified if findings from clinical studies are compared with the results of the above-mentioned model experiment using heart samples of dogs receiving coronary perfusion.

(5) Effects of smoking on the cerebral circulation, especially the speed of arterial blood flow (Kimura K.et al)

This study analyzed the effects of smoking on cerebral circulation using the Doppler method and observed that smoking accelerated transiently the speed of internal carotid artery circulation. The mechanism involved in this was found to have no association with hyperrespiration, the conventionally assumed cause of such acceleration (1983).

However, it is possible that such elevation in the speed of cerebral blood flow is a secondary change following smoking-induced elevations in heart rate and blood pressure, so the acceleration in the blood flow rate does not necessarily indicate an increased volume of blood flow. In this connection, the direct measurement of the cerebral blood flow using this or other methods will be most useful.

(6) The effect of smoking on microcirculation of organs (Abe et al.)

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Using the organ reflectance spectrophotometry developed by the authors, Abe et al. performed a quantitative time-course study on the effects of smoking on the microcirculation of living organs and tissues and on the changes in local oxygen supply and demand. From basic studies on the applicability of this system to the analysis of gastric mucosa and peripheral tissues, they found this system to be applicable to the analysis of changes in local microcirculation as well as to separation and quantitative analysis of both oxy-hemoglobin (HbO_2) and carboxyhemoglobin (HbCO) (1980). Therfore, these researchers found that a reduction in the peripheral blood HbO_2 level and a 5 to 20% elevation in the peripheral blood HbCO level as well as a 22 to 63% reduction in local blood flow occurred during smoking (1980 and 1981). In addition, a comparison between habitual smokers and non-habitual smokers showed that the former had a smaller reduction in blood flow and quicker recovery after cessation of smoking, thus showing the presence of an adaptive phenomenon in hemodynamic behavior in connection with smoking (1981). Further, smoking caused a reduction in the microcirculation in the gastric mucosa of gastric ulcer patients even when they were habitual smokers, thus

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indicating the necessity for additional studies on the effects of smoking and healing of gastric ulcer (1982).

Utilization of this system enables real-time, non-invasive quantitative measurement of the local microcirculation and oxidation in organs and tissues during smoking. In addition, application of the Functional Imaging System (1983), which enables direct display of changes as functional images, will prove to be a highly effective research tool in the analysis of changes during smoking in clinical cases. This system is also applicable to model experimental systems, and thus provides a strong means for studying the effects of tobacco components (nicotine, etc.) on the microcirculation.

3. Directions for future studies

The pharmacologic actions of nicotine are complicated, and the effects of smoking need to be discussed in connection with the additive and off-setting effects of various factors present in complicated organisms. Thus it is difficult to determine the action of smoking on the cardiovascular system and to discuss the significance of smoking as a risk factor. In spite of such difficulties, the following studies are of special importance:

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1) Epidemiological studies

To study epidemiologically the effects of smoking on circulatory disorders in connection with diet, stress, physical exercise and other diseases.

2) Basic studies in experimental model systems

(1) To study the effects of smoking on atherosclerosis using model animals and animal models.

(2) To analyze the effects of smoking on blood flow at the levels of organs and tissues, especially the behavior of cerebral and coronary blood flow.

3) Clinical studies using quantitative measurement and evaluation methods

(1) To study analytically the effects of smoking on blood flow at the levels of organs and tissues and to clarify the role of the autonomic nervous system.

(2) To measure non-invasively oxidation at organ and tissue levels during smoking.

(3) To determine platelet functions during smoking through a rheological approach to blood flow.

In contrast to Europe and America, in Japan the mortality rate from ischemic heart diseases is low, and there is no clear relationship between cerebral apoplexy and habitual smoking. Therefore, the role of smoking needs to be clarified in connection with daily life style, including diet.

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III. SMOKING AND THE RESPIRATORY SYSTEM

1. Significance of studies

It is natural that the first focus of attention in the study of smoking and health was the respiratory system, which is directly exposed to tobacco smoke. Diseases targeted in these early studies were lung cancer, chronic bronchitis and pulmonary emphysema. The ways of approach to problems in these disorders were morphological analysis and respiratory function tests. To date, a large number of reports have been published on these issues. Along with advancements in high technology, it has become possible to approach these issues not only from the point of view of respiratory functions but from that of metabolic and immune functions of the lung. This progress, along with advances in image diagnosis techniques, has enlarged the width and depth of study. Development of these new methods and their application to research are of great significance. The extended coverage of this commissioned study in the respiratory field supports this view.

2. Progress and main results of studies

The project study can be classified into the following three categories: 1) clinicopathological study of habitual

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smoking and lung diseases; 2) study on acute effects of tobacco smoke in healthy individuals and experimental animals; 3) exposure experiments in organisms using nicotine aerosol of regulating particle size. In category 1), clinicopathological study (with the main emphasis on the association with smoking) was performed not only on lung cancer, pulmonary emphysema and chronic bronchitis but on diffuse interstitial pneumonitis, granulomatous lung diseases, diffuse panbronchiolitis and bronchial asthma. In addition, a study on the influences of smoking on the aging process of respiratory functions was initiated during this period. These studies are of great significance in this country where the average age of the population is rapidly increasing. In category 2), study of the acute effects of smoking, the methods applied were not the conventional respiratory function tests (a large number of reports have been published both in Japan and foreign countries using this method), but body plethysmograph technique to measure special airway conductance and He-flow volume curves to measure small airway obstruction were used. Further, a unique study was conducted which analyzed chemical substances in the blood (histamine, serotonin, ACE, fat peroxide, 6-keto

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$PGF_{1\alpha}$, thromboxane B_2, etc.) and complements in connection with smoking in healthy individuals. In animal studies, the effects of cigarette smoke on the central and peripheral airway reactions, pulmonary circulation and systemic circulation as well as the effects on chemical substances in the bood (histamine, serotonin, leukotriene, etc.) were examined in dogs, rats, mice, guinea pigs, sheep, etc.

The acute effects of smoking on the fluid and cellular components of BALF were also studied in animals, and the results obtained were utilized in clinical studies. Also studies on the effects of smoking on antibody-producing capacity and the influence of nicotine on ciliary movements have been started. In category 3), a generator to produce ideal sized aerosol particles was developed to meet the required quantitative and reproducible properties necessary for exposure experiments using aerosol.

The study using this apparatus on the effects of uranine, nicotine, histamine, etc. is to be continued.

1) Clinicopathological studies on habitual smoking and various lung diseases

(1) The relationship between habitual smoking and pulmonary diseases (Homma et al.)

The percentage of smokers in pulmonary diseases

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was as follows; 96.5% in lung cancer, 88.6% to 100% in pulmonary emphysema, 72.7% in chronic bronchitis, 48.8% in bronchial asthma and 40.6% in diffuse panbronchiolitis (DPB). Thus, a high percentage of habitual smoking was seen in patients with lung cancer and pulmonary emphysema. What is noteworthy in the above statistics is that none of the female patients with DPB smoked habitually, suggesting that this disease has little association with smoking. In addition, the presence/absence of habitual smoking did not cause any difference in the parameters of respiratory function either in males or in females, nor was any association observed between such parameters and the cumulative quantity of cigarette consumption. These results suggest that once this disease has developed, the respiratory disorder inherent in it is predominant, and it is difficult to separate this respiratory disorder from the additive effects of smoking. In lung cancer patients, habitual smoking was most common among patients with squamous cell carcinoma followed by cases of small cell carcinoma. Even in patients with adenocarcinoma, the percentage of smokers was larger than in the background population. In patients with pulmonary emphysema, they began to smoke at an average of 20.4 years of age and the average number of years between initiation of smoking

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and development of this disease was 39.8 years.

According to the questionnaires conducted in 1979, 92.5% of the patients with pulmonary emphysema quit smoking after rehabilitation, 80% of them motivated by the education received during hospitalization and 20% by worsening of subjective symptoms, Factors having exerting an influence on prognosis of this disease were %VC, $FEV_{1.0}$, $PaCO_2$ and obesity. No association with the amount of cigarette consumption has been found .

(2) An endoscopic study on the relationship between bronchial atrophic changes and smoking (Kusama et al.)

As the Brinkman index grows, the severity of atrophic changes increases. Atrophic changes were most common in patients with chronic bronchitis, namely noted in 89(67.4%) out of 132 patients. In patients having symptoms of this disease for four or more years, the incidence of such changes was as high as 70.6%; this incidence increased with the lapse of time. In addition, chronic bronchitis cases complicated by primary lung cancer showed a high incidence of atrophic changes (82.4%). A large portion of these cases were male heavy smokers. Histopathologically, hilar squamous cell carcinoma and small cell carcinoma were predominant.

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(3) A study on the relationship between selective alveolobronchographical findings and smoking (Kusama et al.)

Irregular walls of moderate or higher severity were detected at a larger percentage as cigarette consumption grew; heavy smokers showed a tendency to overt shortening of the middle size bronchi during expiration.

(4) A study on the relationship between local morbid pathology measures by broncho-alveolar lavage (BAL) and smoking (Oshima et al. and Homma et al.)

Results of studies using BAL technique, a technique of increasing clinical use since 1976, are presented. According to these studies, smoking caused no significant modification in the elevated total cell number in the BAL fluid or in increased lymphocytes or neutrophils, which are known as disease-specific phenomena of sarcoidosis and idiopathic interstitial pneumonitis. However, in smoking patients showing bloody sputum with no abnormal roentgenogram or having local pulmonary lesions the total cell number in the BAL fluid showed a tendency to increase. In cases of sarcoidosis, chronic beryllium lung,

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hypersensitive pneumonitis, active tuberculosis and interstitial pneumonitis including collagen diseases, the activity of IgG-FC receptors of alveolar macrophages in the BAL fluid showed no difference between smokers and nonsmokers, thus indicating no effect from smoking. In addition, the activity of the C_3 receptor of alveolar macrophages was low evaluated from rosette-forming and phagocytic capacities in all above-mentioned diseases, thus showing an absence of variance among disease categories or between smokers and nonsmokers.

On the other hand, a study on the effects of smoking on the fluid components of BALF in patients with sarcoidosis revealed a decrease in albumin, IgG/albumin and IgA/albumin, and an elevation in CEA(z)/albumin in smokers. The CEA/TP in the BALF was significantly higher in patients with idiopathic interstitial pneumonitis than in control patients.

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This parameter was especially high in many of pneumonitis patients complicated with lung cancer. This parameter was also high in some control patients who smoked habitually. Such a high level of CEA in the BALF may be attributed to the destruction of lung tissues by smoking, causing the contamination by NCA which has cross-reactive property with CEA. Further, fibronectin/albumin was high in control patients who smoked habitually. Fibronectin is widely present in plasma and tissues; it is also found in the peripheral airways. This substance is reported to be formed by alveolar macrophages. Thus, its association with the increase in macrophages paralleling an increase in the total cell number in the BALF of smokers is noteworthy.

(5) Histopathological study on the bronchial epithelium (Homma et al.)

The relationship between smoking and transitional epithelial metaplasia, squamous metaplasia, bronchiolar epithelial hyperplasia and alveolar epithelial hyperplasia was studied in 203 autopsied lungs. In cases with honeycomb lung and interstitial pneumonitis, 12(43%) out of the 28 cases showed squamous metaplasia. In terms of the relationship between squamous metaplasia and smoking, a high percentage of patients having squamous metaplasia

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in the peripheral alveolar region were smokers, whereas a high percentage of patients showing such metaplasia in the bronchial mucosa were nonsmokers. Thus, it was suggested that squamous metaplasia seen in idiopathic interstitial pneumonitis is associated with smoking. This kind of change, however, was not seen in any of the 40 patients with DPB except for the one case showing adenoid metaplasia. This finding is consistent with the afore-mentioned result that habitual smoking had little association with DBP.

(6) Study of the effects of smoking on the aging process of respiratory function(Harasawa et al.)

With growing age, both males and females showed gradual decreases in VC, FVC, $\dot{V}_{50}$, $\dot{V}_{25}$ and MMF. When age-dependent changes in smokers were studied comparatively on the basis of the primary regression formula, changes in male smokers were about 10% greater than those in nonsmokers, while female smokers showed 20% to 40% greater changes than did female nonsmokers. Namely, when examined in terms of respiratory function , the effects of smoking on the aging process are understood to be slightly promotive, and this action is greater in females than in males.

However, no association between early abnormalities in peripheral airway functions and quantity of cigarette consumption could be deduced from this study. In the 1982

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study on age-dependent changes represented by reactivity to ventilating stimulation, it was revealed that reactivity to CO_2 in healthy individuals showed only a slight age-dependent change, and this change was not significantly related to smoking. The effects of smoking on age-dependent changes in the pattern of ventilation were clearly seen in middle-aged individuals (between 40 and 50 years).

2) Acute effects of cigarette smoke in healthy individuals and experimental animals.

(1) Evaluation by respiratory functional studies (Homma et al. and Takishima et al.)

These researches observed smoking-related changes in FVC, $FEV_{1.0}$, peak flow, special airway conductance, Viso $\dot{V}$, etc., in healthy humans and dogs; thus, it was made clear that cigarette smoke can affect not only the central airway but the peripheral airway as well. Also, in experiments with intravenous administration of nicotine, both central and peripheral airways showed a contractive reaction, the mechanism of which was seemingly associated with the vagus nerve, the ganglion and the stimulus-transmitting pathway which causes release of histamine (1983).

(2) A study of the effects on lung metabolism (Homma et al.)

In healthy humans cigarette smoking caused changes in levels of blood histamine, serotonin, ACE activity, 6-keto $PGF_{1\alpha}$, fat peroxide, throm-

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boxane B_2, and so on. These findings showed the influence of smoking on the soluble factors (1980 and 1981).

In an experiment with dogs receiving nicotine via the pulmonary artery, elevations in endotracheal pressure, pulmonary arterial pressure, left atrial pressure and general blood pressure, and reductions in cardiac output and the blood flow of the left pulmonary artery were recorded. For histamine, release of which was presumed in the previous experiment with intravenous administration of nicotine, direct measurement of plasma histamine levels in this study revealed higher concentration after administration of nicotine. In addition, the blood serotonin level was found to reach a peak immediately after administration. Thus, it was concluded that nicotine might be associated with the production and release of various chemical mediators(1979).
In an experiment with guinea pigs exposed to cigarette smoke, a promotive action on the release of histamine, serotonin and leukotrienes was confirmed(1982).

(3) Effects of smoking on the respiratory defense mechanism

a) Until recently there have been no reports published on the relationship between smoking and complements. The first study on this topic was recently conducted, and it revealed that CH50 showed no change at all after smoking, while ACH 50 decreased after smoking. In addition, blood ACH 50 and C_{3c} levels before

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smoking were significantly low in smokers, indicating the presence of alternative pathway activation (Homma et al., 1982 and 1983).

b) Animal experiments: In a compulsory smoking experiment in un-anesthetized sheep, BALF showed an increase in neutrophils. Such an increase was also observed in a one-day inhalation experiment and a seven-day consecutive inhalation experiment (Kusama et al.). Measurement of the fluid components in BALF in rats, revealed an increase in secretion from the bronchial glands including lysozyme, IgA and substance with antitrypsin activities. In a two-week consecutive smoking experiment, however, secretion was reduced for all of these substances. In contrast, IgG showed a transient increase slightly later than the above two substances, showing no change attributable to consecutive smoking (Oshima et al.). This acute effect of smoking promoting secretion from the bronchial gland was also observed in the mice . An additional study was made to determine the influence of smoking on the antibody-forming capacity in mice; in this study, capacity of producing antibodies to sheep red blood cells (SRBC) was examined by measuring the number of PFC in the spleen of mice. It was found that, irrespective of the amount of SRBC, animals smoking for three consecutive days (20 minutes each time) had a smaller number of PFC, thus

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suggesting suppressive action of smoking on antibody-producing capacity (Oshima et al.).

In an in vitro experiment on the effect of nicotine on the frequency of ciliary movement in the airway, no significant change was observed at nicotine concentrations of 10^{-3} M and 10^{-6} M (Harasawa et al.).

3) A study on intrapulmonary deposits and physiological action of nicotine aerosol (Torizuka et al.)

Specific aerosol and its generator have been developed after five-years of research works. This aerosol has favorable particle size, stability and uniform quality good enough for use in exposure experiments. This newly developed aerosol is made of dioctylsebacate(DOS) coated with uranine, nicotine, histamine, etc. It is not hygroscopic. Conditions for generation of 1 μm simple dispersion aerosol are the concentration of the agent at 10^{-2} M, the temperature for evaporation at 150°C, the flow rate of gaseous medium at 1 l/min and the reheating temperature at 190°C. This aerosol carries, irrespective of the kind of drugs, about 100 μg of the agent per liter of air. By adjusting such conditions as the temperature of the boiler, the flow rate of the gaseous medium and the reheating temperature, the aerosol generator can produce aerosol with stable particle size and concentration for several hours.

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This apparatus will contribute to further advance in the study of physiological action of intrapulmonary deposits.

3. Directions for future studies

Conventional approaches to the issue of the relationship between smoking and the respiratory system have been epidemiological, pathomorphological and respiratory-physiological ones. Nowadays, along with the development of better analytical methods for pulmonary metabolism and immuno-defense mechanisms, the study of this issue has become markedly broader and more in depth than ever; it has become multidisciplinary. Therefore, what is required now is to further study the epidemiological, morphological and respiratory-physiological aspects on the basis of past results and at the same time to undertake new research on the metabolic and immuno-defense mechanisms, so that old and new findings can be put together to attain new interpretations and solutions. Generally, the immediate or acute effects of smoking are easy to grasp, so there are many reports published on these effects. However, it is difficult to document the chronic effects of smoking directly, so these effects have not been sufficiently investigated by conventional research. Thus, one of the major tasks from now on is to make up for this weakness.

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IV. SMOKING AND PHYSIOLOGY AND PHARMACOLOGY

[1] BASIC SCIENTIFIC ASPECTS

1. Significance of studies

Smoking is a habit seen only in humans. The strong taste-oriented nature and complicated pharmacological effects of smoking on the function of organisms are chiefly attributable to nicotine contained in tobacco. A large number of pharmacological studies on nicotine have been conducted, since Langley and Dickinson (1889) applied nicotine to the sympathetic superior cervical ganglion of rabbits and demonstrated that its site of action was not nerve fibers but ganglion. However, there are many unknown aspects concerning the action of nicotine on experimental animals. Difficulties in clarifying the action of nicotine are attributable to the following reasons; 1) biological responses to nicotine in a living body emerge as a sum total of separate reactions to this alkaloid in many different tissues and organs, 2) the structure, function and distribution of nicotinic acetylcholine receptors have not been fully understood and, 3) microanalysis of endogenous substances, such as neurotransmitter and candidates being related to the actions of nicotine, was not performed. These problems, however, are gradually being solved due to technological innovations in recent years such as the development of medical electronics and the introduction of immunological assay methods and genetic engineering into the medical sciences.

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2. Progress and main results of studies

1) Effects of smoking on organs regulated by the autonomic nervous system

Many reports already appeared concerning the effects of nicotine on gastic functions. The results of these reports, however, were obtained by the systemic administration of nicotine but not by direct administration of this alkanoid to the central nervous system. Therefore, Osumi et al. studied on this topic. When gastric functions were indexed with gastric acid secretion and mucosal blood flow, nicotine caused centrally mediated dual effects on these gastric functions in urethane-anesthetized rats. When gastric functions were depressed beforehand, nicotine promoted these functions by activation of nicotinic acetylcholine receptors present in the ventromedial hypothalamus. In contrast, when gastric functions were enhanced, nicotine suppressed these functions by releasing noradrenaline from the noradrenergic nerve terminals present in the dorsal motor nucleus of the vagus. A future theme of this study will be clarification of the way how such central actions of nicotine (in sum total with its peripheral action) emerge during smoking. In addtion, it will also be essential to clarify the relationship between these central actions and the physiological role of the brain/gut polypeptides, which have recently been discovered and identified one after another.

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In studying nicotine's effect on the isolated tracheo-bronchial smooth muscles, it was found that the effect of nicotine differed according to regions (trachea, bronchus, etc.) and animal species (Takayanagi et al.). For example, in the guinea pig tracheal muscles, nicotine caused dual responses, i.e., contraction and relaxation. Of these responses, contraction occurred with a relatively low dose of nicotine through the release of acetylcholine. Relaxation, by contrast, was obtained only when a relatively high dose of nicotine was applied, by catecholamine and/or other unknown substance-mediated mechanism. Furthermore, in the bronchial smooth muscles, nicotine caused contraction in a dose dependent manner, and this response was due to a direct action of this alkaloid on the smooth muscles, but not a neurogenic mechanism. On the basis of these findings, they assumed the presence of nicotinic acetylcholine receptors in the smooth muscles. This assumption is interesting, but it challenges a commonly accepted concept. Therefore, further studies are needed to verify such a new concept.

Contraction of bronchial smooth muscles was also produced by nicotine in rabbits. This response was, however, mediated by release of acetylcholine from the nerve terminals, and was different from that seen in guinea pigs. Thus, the response of bronchial smooth muscles to nicotine varies greatly according to animal species. Detailed

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comparative studies are necessary before applying the results of experimental studies to humans.

Recent findings concerning the effects of nicotine on the vascular system are as follows (Muramatsu et al.). The isolated thoracic aorta of rats precontracted by noradrenaline was relaxed by treatment with acetylcholine, but this relaxation was reduced in preparations which were obtained from animals previously received subacute oral doses of nicotine. Moreover, this relaxation was not observed, when the endothelial cells had been previously scraped off from the preparations. Thus, it is expected that the mechanism of action of nicotine will be clarified in connection with the physiological functions of endothelial cells which play an important role in dilation of blood vessels and inhibition of platelet aggregation.

2) Release of endogenous substances such as catecholamines and peptides

It has been reported that nicotine directly affects catecholaminergic nerve terminals (such as the noradrenergic and dopaminergic ones) and releases transmitters not only from the peripheral sympathetic nerves but also from the central nervous system. In previous studies, however, a large dose of nicotine was required to exert this releasing action and the physiological significance of the transmitter release by nicotine remained unclear. In the study of Imura et al., it was found that a relatively low concentration of nicotine (5×10^{-6}M) can promote the release of dopamine from the hypothalamic slices of rats. In addition, an

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increase in the plasma level of prolactin induced by a derivative of methionine-enkephalin was suppressed by nicotine intracerebroventricularly applied in urethane-anesthetized rats. From these results, they suggested that the prolactin release inhibiting action of nicotine was probably mediated by endogenously released dopamine (Imura et al.). These results are consistent with those of Osumi et al. that nicotine-induced inhibition of gastric functions was exerted by release of transmitter from the central noradrenergic nerve terminals.

Effects of systemic administration of nicotine on the turnover rate of catecholamines in the rat brain were studied after inhibition of catecholamine-synthesizing enzyme activity (Misu et al.). In this study, they found a decrease in noradrenaline content only in the hippocampus with a large dose of nicotine 1 mg/kg. In the regions other than the hippocampus, nicotine did not affect the levels of noradrenaline, dopamine and MOPEG (a major metabolite of noradrenaline). Different from the results obtained in in vitro experiments, the effect of the systemic application of nicotine on the levels of catecholamines in the brain seems to be the sum total of the changes induced by a direct action of nicotine on the central catecholaminergic nerve terminals and changes due to other central and peripheral actions of this alkaloid. Hence, to study the metabolism of brain catecholamines during smoking, the results obtained by systemic

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administration of nicotine should be analyzed in reference with the results of the in vitro studies. As related to the mechanism of nicotine-induced release of noradrenaline, Fujiwara et. al. suggested that the release was mediated by Na spike, based on their experimental results in isolated thoracic aorta of guinea pigs.

3) Studies on nicotinic acetylcholine receptors (Fujiwara et al.)

To clarify the central effects of nicotine in detail, it is of importance to know regional distribution of nicotinic acetylcholine receptors in the brain and their molecular pharmacological natures. Previous studies on these nicotinic receptors were performed using alpha-bungarotoxin (a snake venom) as a ligand. Recently, however, it was reported that alpha-bungarotoxin did not always inhibit the nicotinic receptor-mediated biological responses and that the specific binding of the above-mentioned snake venom to cerebral and other membrane preparations was not inhibited by a low concentration of nicotine. Thus,Fujiwara et al.carried out studies using ^{3}H-l-nicotine as a ligand. Summarizing the results so far obtained, the strongest inhibition of the specific binding of ^{3}H-l-nicotine to rat brain preparations was manifested by unlabelled l-nicotine. The second strongest inhibitor was acetylcholine, and next, d-nicotine. Alpha-bungarotoxin was a weak inhibitor. Hardly any inhibition was found with

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hexamethonium or atropine. From these results, it was concluded that the binding site of ^{3}H-l-nicotine was nicotinic acetylcholine receptors, and that this was not the binding site of alpha-bungarotoxin. Furthermore, the possible presence of stereospecificity of the binding site of nicotine was suggested on the basis of findings that l-nicotine exerts about a 100-fold more potent inhibition than does d-nicotine. These results in experimental animals were further confirmed in human brain preparations obtained by autopsy. These are interesting findings and will be of value in elucidating the effect of nicotine and smoking on organisms, and the habitual nature of smoking.

4) Studies on d-nicotine (Fujiwara et al. and Osumi et al.*)

In this series of experiments, stereospecific, pharmacological studies on d-nicotine were performed. An interest in the pharmacological studies on d-isomer of nicotine was partly originated from the following pharmacological properties of d-amphetamine. The central action of d-isomer of amphetamine is much more potent than that of l-somer, whereas the peripheral action of d-isomer is less potent than that of l-isomer. This is also the case for other drugs acting on the central nervous system. Therefore, it would be useful in preventing adverse reactions to smoking, if it is possible to weaken undesired actions of nicotine on the peripheral autonomic nervous system without reducing the favorable psychopharmacological effects of nicotine during

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smoking. However, the pharmacological effects of d-nicotine on the central and peripheral organs were much weaker than those of l-nicotine and there were qualitative differences between pharmacological actions of these two isomers. Results obtained are listed below:

(i) l-Nicotine released noradrenaline by its direct action on the sympathetic nerve terminals and a resultant contraction occurred. When examined in isolated rabbit pulmonary arterial preparations, whereas these effects were not observed with d-nicotine.

(ii) The relative potency of d-nicotine to l-nicotine differed according to organs, tissues and parameters used.

(a) Nicotine stimulated sympathetic cervical ganglion in urethane-anesthetized cats and produced contraction of the nictitating membrane. Such a potency of d-nicotine was about one-fifth that of l-nicotine.

(b) When the elevation of systemic blood pressure in urethane-anesthetized rats was indexed as a parameter, the potency of d-nicotine was about one-tenth to one-fifteenth that of l-isomer.

(c) The potency of d-nicotine to enhance gastric functions by centrally mediated mechanism in urethane-anesthetized rats was about one-hundredth that of l-nicotine (*). This figure is highly consistent with the dose ratio of d-nicotine to l-nicotine in inhibiting the <u>in vitro</u> specific binding

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of ^{3}H-l-nicotine to receptors in the brain membrane preparations. However, the dose of d-nicotine required to induce tonic convulsions in mice was only about 15 times larger than that of l-nicotine (*).

(iii) Both the action of l-nicotine to release noradrenaline from the isolated vascular preparations and the central action of this alkaloid to enhance gastric functions (*) were suppressed by pretreatment with a non-effective dose of d-nicotine or by simultaneous administration of this isomer with l-nicotine. The important finding is that stereospecificity of nicotinic acetylcholine receptors was detected. From this point of view, further detailed analyses of the pharmacological actions of l-nicotine are necessary in comparison with those of d-nicotine.

5) The effects of nicotine on induction of drug metabolizing enzymes and circadian rhythm (Kurogochi et al.)

In recent years, drug interactions are a subject of extensive studies in basic and clinical pharmacology, and the relationship between induction of drug metabolizing enzymes by nicotine and intrinsic predisposition has been studied from the viewpoint of strain and species differences (mouse, rat, guinea pig, rabbit and dog). The data obtained are enormous in quantity and needed to be summarized.

The studies of effects of nicotine on circadian rhythms started recently and conclusive findings have not yet been obtained. The study by Kurogochi et al., however, is a

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new trial and is interesting from the viewpoint of chronopharmacology. At present, analyses are being made on biological parameters which have typical circadian rhythms such as brain serotonin, blood corticosterone, food consumption, water intake etc., and on the enzyme activity of nicotine oxidase. In experiments using rats given nicotine-containing water, nicotine did not exert significant influence on the circadian rhythm of above-mentioned parameters.

6) Biological responses to tobacco smoke components and epidemiological studies on smoking behavior

Examinations of the biological response to tobacco smoke components were, first of all, performed on the effects of NO_2 gas and tobacco smoke on the contents of central neurotransmitters in rats (Kawai et al. and Hasegawa H. et al.). They examined a possible interaction of nicotine and other components of tobacco smoke on brain level of neurotransmitters. These studies are of great value; however, it is difficult to analyze neurochemically the results obtained such as a marked increase in brain acetylcholine during smoking. Since levels of neurotransmitters in the brain are the sum total of synthesized, stored, and released fractions, it is assumed that a mild stimulus such as smoking is probably not enough to cause significant change in the levels of central neurotransmitters. The above-mentioned changes in animals are regarded to be a result of nonspecific stress to

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forced inhalation of smoke, different from active smoking. Smoke inhalation experiments have been considered to be an experimental model resembling to the smoking behavior in humans. The results of the present study, however, suggest that some problems are involved in such forced inhalation of smoke. Consequently, the inhalation techniques for screening the effect of tobacco smoke on the physiological functions, which are easily affected by nonspecific stress, are required to be improved. Although many difficulties will emerge in performing smoke experiments, this problem is of special importance to be solved.

Clinical investigations of the effects of smoking on cerebral blood vessels using the positron CT scanning technique revealed that the morbidity of cerebral infarction of smokers was epidemiologically higher than that of nonsmokers (Torizuka et al.). In this study, a vast majority of the smokers were males, whereas most of the nonsmokers were females. Therefore, the conclusion is based on the assumption that there is no sex difference in the morbidity of cerebral infarction. Such a biased distribution of subjects in each group may be unavoidable in this clinical investigation, since there is a large sex difference in the smoking rate and CT scanning can be performed only in a limited number of subjects. Nevertheless, it is desirable that the population examined is as uniform as possible.

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The results of epidemiological studies on smoking are as follows: When smokers switched from a standard cigarette product to a low-tar, low-nicotine brand of cigarette, they tended to smoke each cigarette more effectively in the first two to three weeks. Four weeks after changing the brand of cigarette, the smoking behavior returned almost entirely to the original level of cigarette utilization (Sacherer). Furthermore, clinical epidemiological surveys to analyze the psychopharmacological aspects of smoking revealed that a main motive of smoking is related with the pharmacological effects of smoking such as sedation, habituation, promoted evacuation, arousal and fatigue removal (Ohara et al.). Smoking of a favored type of tobacco seems to reduce mental tension and irritation, and this may be one of the major reasons why people continue to smoke in their daily life. Further studies are needed in association with the examination of blood level of nicotine in the living body.

3. Directions for future studies

As mentioned above, fairly detailed knowledge has accumulated in this field over the past five years. These findings will be highly valuable in understanding the problem inherent in smoking and health. Further studies are needed to examine the effects of smoking on organisms in relation to pharmacokinetics of neurotransmitters, already known and recently discovered.

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[2] BEHAVIOR

1. Significance of studies

In the field of behavior, the following four topics on nicotine and tobacco smoking were studied: 1) psychopharmacological effects; 2) psychopharmacological factors; 3) pharmacological effects; and 4) interaction with psychotropic agents. Formation of smoking behavior, maintenance of this behavior and the effects of smoking involve complicated mental factors. When the issue of smoking is studied as a theme of life science, much concern is directed to the physical aspects of smoking, and thus a considerable amount of knowledge has been accumulated about these physical aspects. On the other hand, the mental aspects of smoking have not been studied to such large extent, due to methodological difficulties. Thus,there is little knowledge in this area. Because of these circumstances, themes 1) and 2) were studied with a unique method that allowed monkeys to smoke cigarettes or to perform voluntary intravenous administration of nicotine. This study was made to clarify the mental aspects of smoking using a technique of experimental psychopharmacology and to provide knowledge on this aspect to be utilized in dealing with the issue of smoking and health.

The effects of long-term smoking on the human body also need to be studied, not only by way of clinical observation

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and epidemiological survey but they also need to be studied prospectively by experimental approaches. For animal studies with such purposes, it was thought essential to adopt the method of allowing animals to smoke freely for a long period, just like humans. Thus, for the study of theme 3), the experimental method using monkeys was adopted as a trial. In the past, animal studies on the pharmacological action of smoking were for the most part conducted using the direct administration of nicotine. Therefore, it was not confirmed whether the results would be the same if smoking instead of nicotine administration were adopted. This question was studied under theme 3). The nicotine contained in tobacco is mainly l-nicotine, and the conventionally known pharmacological effects of nicotine was for the most part that of l-nicotine. In this respect in studying themes 1) and 3), the pharmacological effects of d-nicotine was examined in terms of both its mental and physical aspects.

In recent years, opportunities to drink alcohol have been increasing, and the opportunity to take psychotropic agents for medical purposes has also been increasing. Nevertheless, it is not well known what pharmacological interaction occurs between these substances and nicotine. Therefore, theme 4) was studied from the viewpoint of determining the effects of smoking in connection with its interaction with other psycho-

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tropic agents.

2. Progress and main results of studies

1) Study on the mental aspects of smoking (Yanagita et al.)

(1) Formation of the smoking habit and factors related to its maintenance

It is well known that when monkeys are allowed to perform intravenous administration of nicotine voluntarily, they take it frequently, suggesting that nicotine has a reinforcing effect. From this fact, it is assumed that the reinforcing effect of nicotine plays a basic role in the formation of smoking behavior and its maintenance in humans. To attest to this assumption, a study was made in which the formation of smoking behavior was attempted by way of letting rhesus monkeys inhale the smoke of cigarettes. So that monkeys did not start smoking through abrupt, forced inhalation of tobacco smoke, they were first subjected to an inhalation training and then to the smoking experiment. In a smoking experiment after simple inhalation training, it was found that monkeys do not inhale the smoke until a sufficient amount of nicotine has been absorbed. Thus, they were subjected to a consecutive inhalation training with a reinforced schedule using an aqueous solution of sugar as a reinforcer. When monkeys were allowed to inhale tobacco smoke after this training, they

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were found to start smoking cigarettes voluntarily, even without the administration of any more sugar solution. Considering that it took a relatively long time to develop voluntary smoking in monkeys and that the number of monkeys showing voluntary smoking was quite small compared to the total number of monkeys subjected to the training, it was thought that the reinforcing effect of nicotine was a prerequisite for the formation of smoking behavior but not enough by itself to cause such formation, and that other psycho-social factors may be involved in the formation of the smoking habit in humans.

When such inhalation training in monkeys was done in combination with a negative reinforcer (i.e., electric shock was given to monkeys if they did not inhale the smoke), it was found that monkeys formed the habit of smoking more markedly than in the experiment using sugar solution only. This finding suggests that we need to take into consideration negative reinforcing factors as well (escape from the realities of life, etc.) in explaining habitual smoking in humans.

It was in the past confirmed by an experiment with nicotine-free cigarettes that nicotine in tobacco smoke plays a key role in maintaining habitual smoking in monkeys. However, cases were also reported in which suspension of smoking did not markedly affect the incidence of smoking behavior (inhalation), indicating that smoking behavior is not

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always maintained by the reinforcing effect of nicotine alone, but that some monkeys maintain it only as a behavioral habit under the influence of some other factors. In such monkeys, the blood nicotine level during free smoking was much lower than that in the other monkeys, indicating that the former inhale tobacco smoke less deeply.

In another experiment to examine the influence of environmental stimuli on cigarette consumption in daily smoking, similarly to the case with electric shock monkeys consumed more cigarettes when the stimuli of turning on and off of a lamp or the sight of other monkeys accommodated in the adjacent cage were presented to them. This finding confirmed that the amount of cigarette consumption is affected by environmental stimuli.

(2) Reinforcing effect of nicotine and its unit dose

To study how much nicotine needs to be administered to give rise to its reinforcing effect, an experiment was conducted using the method of allowing monkeys to perform voluntary intravenous administration of nicotine. This experiment revealed that a unit dose of 15 to 30 μg/kg gives rise to a reinforcing effect of nicotine. This dose level is similar to the one at which monkeys can discriminate nicotine from other substances for its subjective effect. In humans, even when the entire nicotine

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contained in one cigarette is absorbed, the nicotine absorbed due to one puff would be far below 15 μg/kg (on the basis of body weight), thus suggesting that humans are much more sensitive to the subjective effects of nicotine than are monkeys. The issue of reducing nicotine content in cigarettes has been and is being discussed from the viewpoint of smoking and health. Although many smokers are thought to maintain their smoking habit through the effects of nicotine, the idea of reducing nicotine content has a certain limit to the effects, as shown in the above mentioned experiment in monkeys. In the intravenous self-administration experiment in monkeys, the relationship between the unit dose of nicotine and the frequency of nicotine intake in a fixed time was examined. It was found that, contrary to common pharmacological knowledge, changes in unit dose do not greatly affect the frequency of nicotine intake rate in monkeys insofar as the unit dose is not extremely large or small. This finding is noteworthy because it suggests that, within a certain dose range, the frequency of smoking is not much affected by the nicotine content of cigarettes once the habit of smoking behavior has been established.

(3) Anxiolytic effect of smoking

It is generally felt that nicotine works to ease mental frustration or to remove drowsiness. In animal studies on temporal

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behavior or drug discrimination studies, nicotine proved to have both sedative and stimulative effects, suggesting that smoking has an anxiolytic effect. This finding was followed up by a study of smoking monkeys. When the drinking behavior of monkeys was disrupted, the behavior of smoking was promoted on the first day, but on subsequent days the conflict was weakened by the skillful adaptation of the monkeys to the disruption, thus making the effect of smoking unclear. However, the drinking behavior of monkeys showed little change under the condition where monkeys could smoke, while it showed a marked change under the condition where monkeys could not smoke. This finding suggests that smoking has a kind of anxiolytic effect.

2) Study on the physical aspects of smoking (Yanagita et al.)

(1) Experimental study on the influence of long-term smoking

In this study, the effects of long-term smoking on systemic organs in animals were observed. The study brought the following results: (i) by giving a small amount of aqueous solution of sugar to monkeys, it is possible to induce monkeys to engage in relatively stable free-smoking behavior for several years; (ii) in terms of other points, experiments can proceed in accordance with the procedures adopted in the screening of the chronic toxicity of chemical compounds; and

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(iii) when one rhesus monkey (after 2.5 years of smoking) and two rhesus monkeys (after 1 year and 8 months of smoking) were examined hemato-clinico-biochemically and pathologically, all of them showed the presence of anthracosis in the lung but no other noteworthy changes were observed. Thus, an experimental technique for observing the chronic effects of smoking has been established. What is needed now is to perform experiments with a larger number of monkeys for a longer period of time, using techniques like this one.

(2) Pharmacological effects of voluntary smoking

Experiments were conducted on the effects of cigarette smoking on the movement of the digestive tract, the circulatory functions and the electroencephalogram(EEG). This study revealed that smoking caused suppression of the movement of the digestive tract and elevation in the blood pressure and heart rate, and that some rhesus monkeys showed an inhibited pattern of brain waves, showing waves of larger amplitude and smaller frequency. Thus it was confirmed that cigarette smoking can basically induce changes similar to those seen after nicotine administration. However, in smoking experiments (i) the changes seen were less marked than those seen in nicotine experiments; (ii) the digestive tract was most sharply affected by smoking; and (iii) the effect on the digestive tract could be induced to a certain extent even by sham smoking, i.e., breathed without actually

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inhaling tobacco smoke. The last finding can be interpreted in two different ways: namely, it can be understood as an effect of the behavior itself or as a conditioned response to nicotine.

3) The pharmacological action of d-nicotine (Yanagita et al.)

D-nicotine was studied in terms of its physical effects (such as acute toxicity in mice; effects on blood pressure, tachyphylaxis and heart rate of rats; effects on the removed phrenic neuromuscles and intestines of rats; and effects on EEG of monkeys) and behavioral effects such as hyperactivity of rats, DRL (differential reinforcement of learning) activity of rats and voluntary intravenous administration behavior of monkeys. Through these studies, d-nicotine proved to be qualitatively similar to l-nicotine both in terms of physical and behavioral effects, with the potency of d-nicotine being about one-fourth to one-sixteenth that of l-nicotine.

4) Study on the interaction between smoking and psychotropic agents (Yanagita et al.)

To clarify this issue, the pharmacological interaction between nicotine and alcohol or various other psychotropic agents was studied in animals. The most noteworthy finding

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obtained from this study was that nicotine reinforced the excitatory behavior induced by other drugs. Namely, nicotine markedly enhanced the excitatory effect of d-amphetamine in the DRL experiment and converted the sedative effect of diazepam on the fighting mouse into a excitatory effect. Alcohol generally exerts antagonistic effects to nicotine, but when alcohol was simultaneously given to monkeys that took nicotine by self-administration, some monkeys showed enhancement of the drug-seeking behavior for nicotine, while others showed weakening of such behavior, thus suggesting that alcohol may enhance or depress smoking behavior depending on the situation.

3. Directions for future studies

1) Psychological usefulness of smoking

Studies so far conducted indicate that smoking may result in useful psychopharmacological effects and that these effects may constitute key reasons why people are fond of smoking. Thus, it seems important to clarify significance of these effects in a daily life context by performing aminal studies under practical situations, as illustrated by the ongoing experiment on mental conflict.

2) Factors influencing the amount of cigarette consumption

In daily life, the amount of cigarette consumption seems to be influenced by the amount of nicotine intake, by the

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relationship between nicotine consumption and the comfort/discomfort of its effects and by the internal/external environmental conditions of smokers. Therefore, these factors need to be subjected to psychopharmacological analysis.

3) Relationship between the kinetics of nicotine absorption during smoking and its pharmacological effects

The blood nicotine level during smoking is thought to be composed of a combination of quick transient changes and time-course accumulation. This combination is thought to govern the pharmacological action and affect the frequency of smoking and the depth of inhalation. However, the details involved in this process are not yet well understood. Thus, it seems necessary to clarify the relationship between blood nicotine levels on the one hand and the mental and physical effects of smoking and smoking behavior on the other as well as the relationship between the tachyphylaxis and the saturation of effects, by menas of undertaking experiments in smoking monkeys and humans.

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V. SMOKING EFFECTS ON PREGNANT WOMEN OR FETUSES, AND ON ENDOCRINE SYSTEM AND METABOLISM

[1] PREGNANT WOMEN AND FETUSES

1. Significance of studies

Obstetrical interest concerning the smoking effects on pregnant women has been directed mostly to the effects on fetal growth in utero. Epidemiological study on this problem was first reported by Simpson (1957), and since then, more than 100 epidemiological surveys have been carried out on this issue in North America or in European countries. Most of these works reported lower birth weight among babies born to smoking mothers as compared to those born to non-smoking mothers. In contrast to these studies, several investigators, using matched-controls, described that there was no difference in birth weight between the two groups. Also, there were several epidemiological studies which suggested that cigarette consumption at a level of ten or less per day did not affect fetal growth in utero. Recently, attention was also given to this problem in our country. Therefore, significance of our commissioned study in this field is concerned to the reproductive biology investigating clinical or experimental effects of smoking and/or nicotine against pregnant uterus, placenta or fetus.

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2. Progress and main results of studies

1) Clinical studies

Tojo et al. (1979 and 1980) studied smoking effects on the placental function, using such parameters as urinary estriol, serum hPL, heat-resistant ALP, LAP, LDH, β-glucuronidase, AFP, Cu or "DHA-S load test". This study revealed that

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the levels of above parameters in mild-smoking mothers (20 or less cigarettes per day) were all but identical with those in non-smoking mothers, while heavy-smoking mothers (more than 21 cigarettes per day) were found to have decreased levels of both urinary estriol and serum hPL. Also, a slight increase of estradiol was noted in heavy-smokers after the injection of DHA-S. In this study, it was also indicated that neonates born to heavy-smokers have significantly lower birth weight as compared to those born to non-smokers, while a significant difference was not seen in the weight of placenta or Apgar score.

Morphological change of the placenta obtained from smoking woman was studied by Tojo et al. (1979). According to their study, microscopical change was not found in the placenta obtained from non-smoker or mild-smoker, while placenta obtained from heavy-smoker showed atrophic change in the villi together with hypovascular patterns.

Clinical studies concerning the smoking effects on human fetus were carried out by Tojo et al., Mochizuki et al. and Ishiguro et al.. Tojo et al. (1981) estimated both fetal movement and fetal heart rate after smoking two cigarettes for 15 to 20 minutes in four pregnant mild-smokers, and reported that remarkable change was not seen in two of four cases, while decrease of temporary tachycardia after fetal movement was found

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in the remaining two of four cases (one developed to bradycardia later). Similar study was also done by Mochizuki et al. (1982). These investigators reported that change of fetal heart rate was found in seven of 31 (22.6 %) pregnant women after smoking two cigarettes. Ishiguro et al. (1982 and 1983) also studied serial change of fetal movement or fetal heart rate in seven mild- or moderate-smoking mothers during the smoking of one nicotine-free cigarette and one nicotine-containing cigarette alternately. In this study, maternal peripheral O_2 (pO_2) was also examined transcutaneously. In six of seven women, remarkable change was not observed in both transcutaneous pO_2 and fetal heart rate, while in a patient with placental dysfunction, temporary bradycardia was found during the smoking of nicotine-containing cigarette. These investigators examined, using modified method of gas chromatography mass-spectrometry system (GC-MS), serum nicotine or cotinine level in this woman to determine whether or not the temporary bradycardia induced by nicotine-containing cigarette was due to the effect of nicotine, and they found that both serum nicotine and cotinine were at the same level as in non-smokers. Therefore, these investigators speculated that temporary bradycardia found in this case was not due to nicotine but to other unknown factor(s).

Using cardiotocography, Ishiguro et al. (1982 and 1983)

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also studied the smoking effects on uterine contraction at a late stage of pregnancy, and found that uterine contraction was not induced by smoking, at least, one cigarette.

Biochemical studies were also done by Ishiguro, et al. (1979, 1980 and 1981). These investigators examined various biochmeical components in umbilical cord sera (total protein, A/G ratio, triglyceride, cholesterol, GOT, GPT, LDH, LAP, ALP, ZTT, TTT, bilirubin, creatinine, urea nitrogen, uric acid and vitamin B_{12}) from the neonates born to mild- or moderate-smokers (20 or less cigarettes per day), and reported that significant difference was not noted in any of the above biochemical components between the neonates from smoking mothers and those from non-smoking mothers. In this study, they also studied duration of pregnancy as well as birth weight, however they could not find any significant difference in these parameters between non-smoking mothers and smoking mothers.

2) Experimental studies

(1) Effect of nicotine on human placental cell cultures

In vitro study was done by Sawaragi et al. (1982). In this study, placental tissue obtained immediately after delivery was shortly incubated in culture medium containing nicotine or cotinine, and serial changes of prostaglandin in the culture fluid were examined by radioimmunoassay. A similar study was

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done by these investigators (1983) using chorionic villi obtained at an early stage of gestation. Both studies revealed that nicotine suppressed the production of prostaglandin derivatives, and that the suppression was predominant in the production of PGI_2 or PGE_2 that had prominent vasodilative effect. It was also shown that suppression of the prostaglandin production by nicotine was more apparently induced at an early stage of gestation as compared to a late stage of gestation. Sawaragi et al. (1981) also studied the effects of nicotine or cotinine on prolactin production in vitro by decidual tissues at 5—9 weeks of gestation, and reported that production of prolactin was induced by nicotine, while it was suppressed by cotinine. Although the significance of these findings is not clear at present, it is suggested by these investigators that nicotine may react directly to placenta.

(2) Animal experiments

To study the smoking effects on animal fetus, Ide et al. (1981) exposed pregnant rats to tobacco smoke, and examined the numbers of corpus luteum, implantation, live fetus, dead fetus and absorbed embryo. These investigators reported that there was no difference in such numbers between exposed rats and non-exposed controls. It was also found that birth weight, birth height, tail

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length, weight of the placenta or incidence of major or minor anomaly was almost identical in both groups.

Matsuura et al. (1982 and 1983) studied the effects of nicotine on uterine circulation using pregnant ewe or uterine artery of pregnant sow. These investigators found that nicotine induced the vasoconstriction of pregnant ewe uterus in vivo, being mediated by α-sympathetic stimulation, and that decrease of uterine blood flow induced by vasoconstriction was suppressed by prostaglandin I_2 derivatives or conjugated estrogen. Similar findings were also obtained from in vitro study using uterine artery of pregnant sow, and these investigators suggested that α-sympathetic stimulation might be due to catecholamine release by nicotine.

Takayama M.et al. (1982 and 1983) studied the effects of nicotine on brain growth using fetal or neonatal rats. 2-Deoxy-d-glucose (2DG), being a parameter of glucose uptake in the brain, was labelled with ^{14}C, and cerebral ^{14}C uptake was determined by an autoradiographic method. These investigators showed that there was no difference in cerebral ^{14}C uptake between fetuses of nicotine-injected rats and control fetuses. They concluded from these findings that glucose consumption at the site of regional brain was not suppressed in the fetus that received nicotine trans-placentally. Similar findings were also obtained from the study

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using the neonates born to mother rats treated with nicotine per os during both gestation and lactation. Continuous administration of nicotine per os to pregnant rats or newborn rats was found to cause slight decrease in the uptake of ^{14}C-2DG at whole brain in newborn rats, while increase of ^{14}C-3DG uptake, i.e. increase of glucose consumption was noted in certain regions of the brain. The significance of this finding is still unknown, but is now being investigated by these investigators.

Experimental study using rats was also done by Mochizuki et al. (1983). Pregnant rats were divided into three groups according to the concentration of nicotine injected subcutaneously, and effects of nicotine on the metabolism of both sugar and lipid in mother rats or fetuses were studied. In this study, decrease of both hepatic glycogen level and insulin-binding capacity in adipose tissues was found in nicotine-exposed fetuses. Decrease of serum triglyceride level, hepatic glycogen level and insulin-binding capacity in adipose tissues was also observed in nicotine-treated mother rats. However, fetal serum triglyceride, free fatty acid or glucose in nicotine-treated rats was at the same level as normal controls. These investigators also reported that intrauterine death or major anomaly was not found in the fetuses born to nicotine-treated mother rats. Although there was no difference in the number of neonates between nicotine-treated mother rats and normal controls, significant decrease of both birth weight and placenta weight was noted in the mother rats that received high concentration of nicotine.

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Sawaragi et al. (1979) investigated the ultrastructural changes in placenta or umbilical cord derived from nicotine-treated pregnant mice, and reported that special change was not induced by nicotine.

3. Directions for future studies.

The studies conducted in this field presumably cover all problems that are socially relevant. In Japan, however, female smoker is not so common as in North America or European countries, and also number of tobacco consumption in female smoker is mostly less than 20 per day. Therefore, clinical study in human is restricted to do only in a small number of subjects, and this has often yielded inconsistency concerning the obstetrical effects of maternal smoking. Accordingly, accumulation of clinical findings obtained from a large number of female smokers (particularly from heavy-smokers) shall be required in future. Since the most important purpose of these studies is to elucidate obstetrical or neonatological influences by maternal smoking, clinical studies using human subjects may be more desirable than experimental studies using animals.

[2] ENDOCRINOLOGY AND METABOLISM

1. Significance of studies

Various ideas have been proposed to explain the effects of smoking on various organs in the body, but such speculation has

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not been fully documented in those other than the cardiovascular and respiratory organs. However, a marked progress has been made in the fields of endocrinology and metabolism, with the development of new hormone assay techniques in recent years, although results have not been clearly demonstrated.

Methods for studying the effects of smoking can be grouped into two types: one the effects of smoking itself, and the other the effects of alkaloid (nicotine) contained in tobacco. Nicotine has been accepted a great deal of academic attention because it binds the nicotinic receptor of acetylcholine (one of the neurotransmitters) and promotes the cholinergic mechanism involved in neurotransmission. Since it has recently been found that this nicotinic receptor is involved in the secretory mechanism of various hormones, attention is now paid to examine the relationships between nicotine and hormone secretion in this field. Thus, most studies performed on the effects of smoking on the endocrine and metabolic system have been conducted by means of nicotine administration, whereas only a few studies screening the effects of smoking itself have been reported. In reviewing foreign studies in the endocrine field, it has been demonstrated that smoking induces elevation in antidiuretic hormone, growth hormone, norepinephrine and epinephrine. In Japan, this kind of study has been done only by Imura et al., who have examined the effects of smoking on pituitary, adrenal medulla, pancreatic and

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gastrointestinal hormones. In terms of the effects on metabolism, on the other hand, studies of diabetes (a representative metabolic disease) are unexpectedly few. Although the possible hazards of smoking on diabetes are widely discussed, scientific study on this subject has not been sufficient. The effects of smoking on lipid metabolism have been studied in details, probably because this kind of study is important for the cardiovascular system. Therefore, studies of the effects of smoking on the endocrine function and metabolism lag far behind other fields. It seems to us the keen necessity of promoting studies in this field.

2. Progress and main results of studies

1) Study of the effects of smoking and cessation of smoking on the human endocrine function (Oyama et al.)

Oyama et al. have examined the effects of smoking and its cessation on endocrine functions in healthy habitual male smokers. The study has revealed that smokers show higher levels of plasma cortisol, prolactin and epinephrine, compared to nonsmokers. Beta-endorphin, prolactin and epinephrine levels decreased significantly during refraining from smoking, and when smoking was resumed after cessation a transient elevation in plasma cortisol, beta-endorphin and prolactin levels was noted. This study seems interesting in such an aspect that it aimed at determining the effects of smoking on three hormones secretion simultaneously. There seem to be, however, some methodological problems in this study.

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Measuring time-course changes of such hormones as ACTH and cortisol which show considerable diurnal rhythms requires special attention. More careful studies with control experiments and with stimulation test must be done to draw conclusion. Another problem is a rather unusual way of smoking (two cigarettes for ten minutes in the morning, followed by four hours cessation and the subsequent smoking of two cigarettes) compared with ordinary smoking (i.e., four to six cigarettes in four hours). The difference in the speed of smoking or the stress caused from the prohibition of smoking might greatly affect the results.

2) Study of the relationship between smoking and osteoporosis (Fujita et al.)

Since osteoporosis is a disease which increases greatly from now on as the average age of the population increases in Japan, as elsewhere, it is important to study the effects of smoking on this disease. In this study, what was first examined as basic research was the effect of nicotine on the bone and cultured cells of the kidney (the side of production of active vitamin D which is closely related to osteoporosis). It was found that smoking did not affect cellular proliferation in the bone or kidney, but that it suppressed transport of electrolytes and water. The smoking history, mineral metabolism and presence/absence of bone fracture were also examined in patients with

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osteoporosis, but no difference was noted between smokers and nonsmokers in osteoporosis. In the future, the relationship between smoking and this disease should be further clarified by analyzing the vitamin D metabolites and the bone in smokers in comparison with those in nonsmokers.

3) Study of the effects of smoking on pancreatic functions (Imura et al.)

Epidemiological surveys in recent years have pointed out that the incidence of chronic pancreatitis and pancreatic cancer is high in smokers. In light of this, it is quite important to elucidate the effects of smoking on pancreatic functions. Since the conventional tests for pancreatic exocrine functions are quite complicated, however, the effects of smoking on pancreatic exocrine function have not been examined yet, therefore, pancreatic function tests utilizing endocrinological techniques have been developed. Based on the fact that a new hormone called pancreatic polypeptide is present in the pancreatic exocrine tissue in addition to islets, Imura et al. have demonstrated that pancreatic functions can be examined by measurement of this hormone. They further have indicated the possibility that smoking may have an acute suppressive effect on the pancreatic exocrine functions, since pancreatic peptide is suppressed during smoking. When the pancreatic exocrine function of habitual smokers was evaluated with the pancreatic function diagnostant (PFD) test, it turned out that subjects with a long history of smoking showed lower pancreatic exocrine function, compared to shorter one and non-smokers. This finding is quite interesting, but, since long-term smokers may also be affected by

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factors other than smoking (e.g., alcohol intake, type of food) and may be influenced by latent complications from metabolic diseases, it is necessary to sufficiently survey the background of subjects and to study the effects of smoking on pancreatic exocrine function in patients with metabolic diseases as well.

3. Directions for future studies

As stated above, detailed studies on the effects of smoking on the endocrine function and metabolism were initiated only in recent years, so that many points remain unclarified. However, due to marked progress in endocrinology in recent years, we can expect that studies on the effects of smoking will be more detailed in the near future. In such studies, not only basic experiments using nicotine administration but also screening of the effects of smoking itself are required. Furthermore, smoking has been discussed as a risk factor in diabetes (a representative metabolic disease) in addition to obesity. However, in reviewing the foreign literatures, studies on this aspect of smoking are not clear at present. In Japan, the number of diabetic patients is estimated to be 4 to 6 million including potential diabetic patients. In the population above the smoking age, diabetic patients account for about 6% to 8%, a particularly high percentage. Thus, it is an urgent necessity to clarify the effects of smoking on diabetes as well as on osteoporosis. It is no exaggeration to say that virtually no studies have been performed on the effects of smoking on these diseases. Therefore, we should initiate studies on these aspects without delay.

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VI. PASSIVE SMOKING

1. Significance of studies

In Japan, increasing attention is being paid to the effects of passive smoking. Studies on this theme, however, have only a short history and results are scant. In addition, there are many methodological problems in this kind of study. Under the present circumstance , it is of great importance to conduct well planned studies to obtain scientifically correct evaluations and conclusions.

2. Progress and main results of studies

1) A study on the development and application of an analytical technique to determine indoor air pollution by cigarette smoke (consisting mainly of trace amounts of various chemical compounds) (Matsushita et al.)

How to separate and measure various polycyclic aromatic hydrocarbons in indoor air was studied. They

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developed a simple, highly sensitive quantitative analytical method for benzo(a)pyrene through the use of high-speed liquid chromatograph equipped with a spectro-fluorescence detector and a highly sensitive simple analytical method for aromatic nitroso compounds through the use of glass capillary gas chromatograph . With these new methods, they found that the chromatographic pattern of these compounds differed according to cigarette brands (1979 and 1980). They further established a method of measurement (composed of a liquid-solid capturing system and a gas chromatograph with a thermal energy detector attached) for quantitative analysis of N-nitrosoamine (a carcinogenic substance released into the indoor air by smoking) by separating it from various artifacts. By applying this method to measurement of the N-nitrosodimethylamine and N-nitrosopyrrolidine contents in the sidestream of cigarette smoke of 15 different domestic and foreign cigarette brands, it was disclosed that the contents of these substances varied greatly according to brands. It was also found that these substances could be detected in offices or coffee shops where people smoked, and that air pollution from these substances could also occur through air polluting factors other than smoking (1981 through 1983).

2) A RIA study on acute and chronic effects of passive smoking by measurement of urinary nicotine, cotinine and CEA (Matsukura et al.)

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Matsukura et al. established a radioimmunoassay (RIA) method for microanalysis of urinary cotinine and found that the amount of cotinine closely paralleled the amount of cigarette consumption. Therefore, using this method, they studied passive smoking.

In an experiment in a minibus where subjects were acutely exposed to the smoke of six cigarettes (non-filtered "Peace") for two hours, nonsmokers showed no significant elevation in their urinary cotinine and nicotine levels irrespective of the presence/absence of ventilation (1979).

When a smoker(s) who consumed 21 cigarettes or more per day was present, nonsmokers in the same home showed a significant elevation in their urinary cotinine level. Even when there was no smoker present in the home, individuals living in urban areas showed higher levels of urinary cotinine than did those in rural areas(1980).

When there was one or more smokers in the same home or office, the urinary cotinine level of nonsmokers increased with the number of cigarettes consumed, but urinary carcinoembryonic antigen(CEA) level showed no definite correlation(1981). The urinary CEA and calcitonin levels of nonsmokers were found to be unaffected by passive smoking at home or at office (1982). Young children, who were most susceptible to the influence of smoking at home, showed an elevation in urinary cotinine level, depending upon the total quantity of cigarette consumption of family members,

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especially consumption at home. In this context, influence from the mother's smoking was particularly large (1983).

3) Survey questionnaire on respiratory symptoms, smoking status at home and family environment and an investigation of chronic passive smoking using the $\dot{V}$-V curve test in school-age children in districts with different severities of air pollution (Tsunetoshi et al.)

The incidence of asthmatic bronchitis in young children was high in districts where air pollution was severe or in families whose members smoked habitually (1979). The results of this 1979 study were later reconfirmed. In addition, it was found that air pollution in homes using non-ventilating room heaters caused an elevation in the morbidity index of asthmatic symptoms, and that the morbidity index of respiratory disorders among school-age children was elevated by the additive or synergistic effects of indoor air pollution, outdoor air pollution and passive smoking. This incidence was highest in the group where mothers smoked at home, the second highest when grandfathers or grandmothers had such a habit, and the third largest when the father smoked. The incidence was lowest in homes where nobody smoked. The effect of air pollution, however, was distinctly larger than overt effects of passive smoking. The degree of NO_2 exposure in individuals was based mainly on the degree of home air pollution and much less

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on the factor of passive smoking. A study on asymptomatic school-age children revealed that there was no overt relationship between the degree of passive smoking at home and respiratory function data (1981 and 1982). In children with strong predisposition to respiratory disorders (e.g., high IgE level), extrinsic factors (passive smoking, air pollution, etc.) have a smaller share in the morbidity index of respiratory disorders than in children without such a predisposition (1983).

4) Survey on chronic passive smoking using the ratio of urinary hydroxyproline to creatinine (HOP ratio) as a parameter related to collagen metabolism (Kasuga et al.)

In school-age children living in districts with different severities of air pollution, the HOP ratio in urine samples (regarded as a parameter of destruction and repair in the lung structural protein, i.e., collagen) was examined, and the children were classified according to the status of passive smoking within the family. The HOP ratio paralleled the number of consumed cigarettes, but it increased with growing cigarette consumption at home and growing severity of air pollution. In addition, the ratio was related to air pollution at home, such as the use of non-ventilating room heaters. Thus, it was strongly suggested that the increase was attributable to latent pulmonary

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destruction caused by oxidants (factors commonly found in these cases), particularly NO_2 (1981). The urinary HOP levels in school-age children and their mothers increased with increased consumption of cigarette by their fathers, and the increase in mothers was more distinct. This effect on children became more well-defined when their mothers were smokers. The correlation between the HOP ratio on the one hand and individual NO_2 exposure and the number of cigarettes smoked passively on the other was significant, but there was no significant association between individual NO_2 exposure and the number of cigarettes smoked passively. In both winter and summer, individual NO_2 exposure is higher in mothers than in children, due to exposure to NO_2 in the kitchen. School-age children, in contrast, receive much higher exposure to NO_2 from automobile emissions than do their mothers from NO_2 in the kitchen (1982 and 1983).

5) Survey on passive smoking by measuring the nicotine concentration in ordinary indoor air (Kira et al. and Matsushita et al.)

In ordinary environments, nicotine in the air was collected by means of passing the indoor air (about 1 l/min) through a Uniport-KA collector (a carrier made of acid-treated diatomite). After adding an internal standard (isoquinoline), nicotine was extracted by alkaline methylalcohol and quantitatively analyzed with a flame ionization detector attached to gas

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chromatograph. In this investigation, the average level of nicotine detected in the office, patients' waiting area and dispensary in a hospital was 6 to 15 $\mu g/m^3$. Over time, the level showed a fluctuation of ± 2 SD, depending on the presence or absence of hospital staff, no-smoking regulations for certain time periods, and the number of outpatients and visitors. Nicotine was also clearly detected early in the morning and late in the evening, presumably due to influences remaining from the previous day. In automobiles, the level of nicotine varied greatly according to degree of ventilation and whether the window is open or closed. When there was no ventilation and the window was closed, the smoking of two cigarettes in 20 min led to a high nicotine level of 240 $\mu g/m^3$. At ordinary parties, the level of nicotine contamination was 90 $\mu g/m^3$. The nicotine concentration in ordinary environments fluctuates greatly according to ventilation, room size, cigarette consumption and the traffic of people in and out of the room (1981 through 1983).

3. Directions for future studies

Within the framework of studies evaluating air pollution, study 1) was directed to the theme of passive smoking, whereby the chief purpose of the study was to establish a practical method for quantitative analysis of various chemicals in indoor and environmental air, particularly mutagenic ones. This was

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an important basic study. From now on, it is hoped that simpler measuring techniques for more specific parameters of passive smoking will be developed.

The issue of passive smoking was approached in study 2) by the parameters of urinary cotinine and tumor markers, in study 3) by the parameters of respiratory symptoms and functions, in study 4) by HOP ratio and serum tumor markers using the same epidemiological method as that adopted in study 3), and in study 5) by the parameter of nicotine contamination in indoor air.

Studies 2) through 5) have resulted in noteworthy findings. However, since epidemiological parameters, HOP ratio and tumor markers are not specific parameters and can be affected not only by passive smoking but by environmental and indoor air pollution as well, it is necessary to make a concerted effort to develop specific screening of the effects of passive smoking from the changes detected. In addition, it is also a difficult problem as to how we can reasonably extract the factors involved in passive smoking from environments where key influencing factors such as outdoor and indoor air pollution overlap with each other.

In this sense, urinary cotinine level and indoor air nicotine concentration are specific parameters for the analysis of passive smoking. The former, however, is an index

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of the change in components absorbed and metabolized in the body, while the latter is an index of change in the nicotine concentration in the environment surrounding the individual. Thus these two parameters have different aspects. Cotinine is characterized by its slow excretion, enabling us to grasp the sum total of smoking over a given length of time. Fluctuation of tobacco smoke in indoor air is great due to the influences of various factors; besides, individuals move around in this environment at random. The contact of individuals with tobacco smoke is quite complicated, and its analysis is not easy from the above-mentioned parameters alone. Thus, from now on it will be necessary to study the contact of individuals with tobacco smoke, the bioaction of unabsorbed tobacco smoke components and the effects of absorbed components from an overall point of view.

The above is a summary of the studies on passive smoking. An issue left open for future study is how to establish a methodology to determine the effects of chronic passive smoking on the body. Tabacco smoke in indoor air changes dynamically, and passive smoking is thought to have fairly long-term influences on living organisms. In evaluating this subject, we need specific parameters that can be measured easily and techniques to detect specific reactions in living organisms. These and other difficult basic problems lie on the road toward clarification of the effects of chronic passive smoking on living organisms.

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VII. PSYCHIATRY

1. Significance of studies

In light of its concern with psychosomatic medicine, psychiatry, cerebrophysiology and psychopharmacology, this research group held a joint conference in 1979 and prepared a questionnaire to determine how to proceed with the study of the effects of tobacco and other substances on mental and physical health.

2. Progress and main results of studies

1) Studies by Okonogi et al.

In 1980, Okonogi et al. performed a psychological survey (using three survey forms, A, B and C) on three groups (smokers, ex-smokers and nonsmokers) and obtained various findings about the motives for smoking, the age when smoking was initiated, the situation of subjects at the time when smoking was initiated, the effects of smoking on the mind and body, the motives for quitting smoking, the effects of cessation of smoking, the view of subjects about the effects of smoking on health and the general relationship with other favorite foods. In addition, through individual interviews with psychiatrists and psychologists, they obtained

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a working hypothesis.

In addition, they performed a survey using the Cornell Medical Index (CMI) from 1981 to 1982, and found the male ex-smokers are apt to show a tendency towards neurosis. Through item-by-item analysis of the CMI data, they were able to confirm that smokers have more widespread and specific subjective symptoms in the respiratory system, digestive organs and bones or muscles than do nonsmokers. In addition, although there was hardly any difference between smokers and ex-smokers concerning knowledge of the effects of smoking on health, ex-smokers proved to feel neurotic unrest more strongly than smokers, indicating that this feeling of unrest is a key factor among ex-smokers who do not resume smoking.

In 1983, Okonogi et al. summarized their findings (mainly those obtained from individual interviews) and noted the following factors based on the results of their analysis:

(1) Factors which induce smoking: feelings of psychological well-being; oral satisfaction; recovery of one's feelings and perceptions; and feelings of emancipation.

(2) Factors which lead to habitual smoking: binding of the pharmacological action of nicotine and other agents to psychological factors.

(3) Mental and physical reactions of smokers in the nonsmoking state: elevated frustration due to unsatisfied

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oral cravings; unstable emotion and high reactivity to stimulation; loss of self-sensation and feeling of emptiness; helpless feelings caused by loss of the means to resolve these problems.

(4) Analysis of factors leading individuals to quit smoking and to avoid resumption of smoking: (i) knowledge of the hazards of tobacco; (ii) experiences which potentiate such knowledge (unusual physical sensations, disease, death of others from cancer, heart disease, etc.) and life cycle, especially the mental attitudes of middle-aged or elderly people); and (iii) the relationship to personality (persons who are apt to feel neurotic unrest more strongly than others).

(5) Mental processes after the cessation of smoking: (i) mental and physical reactions (unsatisfied oral cravings, instability accompanied by frustration, reduced intellectual and mental activity, changes in self-sensation); (ii) purposelessness; and (iii) changes in social adaptability and in mental and physical rhythms.

2) Studies by Ishikawa et al.

In 1980, a study on the relationship between mental and physical relaxation and smoking was conducted from the standpoint of psychosomatic medicine. Using the parameters of myoelectric potential and cutaneous temperature, which reflect

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the state of relaxation, this study revealed that smoking promotes muscular relaxation.

In 1981, by means of autonomic training and electromyographic bio-feedback techniques, electromyograms during smoking were compared with each other.

In 1982, through a study on the relationship between smoking and relaxation in which the effect of smoking behavior on electromyograms was eliminated, it was confirmed that the relaxing effect of smoking is reflected in these parameters. In addition, when 4,042 healthy individuals were classified by egogram pattern, it was revealed that the group of smokers showed a high percentage of FC ("free child"), while the group of nonsmokers had a high percentage of AC ("adaptive child").

In 1983, using the same survey data, an egogram-pattern analysis was conducted on the nonsmoking group, ex-smoking group, mild smoking group (less than 20 cigarettes a day) and heavy smoking group (20 or more cigarettes per day).

A characteristic finding obtained from this study was that the nonsmoking group showed egogram patterns of "critical parent" and "reasonable ego" at a high percentage, and that subjects of this group can be considered to be persons who are critical and authoritarian and have the ability to grasp reality, in other words, a type of perfectionist.

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3) Studies by Hasegawa K. et al.

Hasegawa K.et al. studied by physical effects of smoking from the viewpoint of tobacco addiction. In 1980, they examined the urinary catecholamine level of subjects to analyze the characteristics of habitual smokers in terms of their amine levels in an attempt to clarify the somatic aspects of tobacco addiction. Among other results, they found an important sign of somatic tobacco addiction in the phenomenon that urinary norepinephrine level changes when those addicted to tobacco cease smoking.

In 1981, they clarified urinary catecholamine elimination for mild smokers and heavy smokers during cessation of smoking and during free smoking.

In 1982, it was found that compulsory cessation of smoking causes mental and physical stress in those addicted to tobacco, and that urinary amine metabolism during compulsory nonsmoking in these people shows a change similar to that seen in withdrawal from alcohol. It was further demonstrated that cerebral amine metabolism changes when acute administrations of nicotine are given. From these findings, the smoking behavior of those addicted to tobacco was considered to be attributable to the nicotine-induced changes in cerebral amine metabolism. Further, it was indicated that chronic nicotine administration does not have any influence on the central

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activity of the hypothalamus, which regulates food and water consumption.

The 1983 study examined the effects of smoking on the hepatic clearance of haloperidol and chlorpromazine in patients with schizophrenia. In this study, no noteworthy change was seen in haloperidol, while with chlorpromazine, the plasma neuroleptic level was reduced in smokers who consumed 20 or more cigarettes per day, suggesting that the activity of chlorpromazine metabolizing enzymes is potentiated by smoking. Thus, this study indicated that certain care should be taken in administering chlorpromazine to patients who smoke 20 or more cigarettes per day.

4) Studies by Chiba

The main theme of this ongoing medical investigation about the mental effects of addictive oral substances including tobacco is to provide a basic theoretical model.

Chiba proposed a hypothesis called the 'SD/SS theory," which attempts to understand human behavior from the following viewpoint:

"The natural tendency of human existence is understood as a dynamic system (SD), whereas humans need to adapt themselves to a static system (SS) to become social beings".

On the basis of this theory, he tried to understand behavior in terms of physiological functions, different from the

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conventional method of explaining behaviors in connection with psychological effectiveness.

From this viewpoint, he worked on constructing the theoretical basis of the SD/SS theory from 1980 through 1983. In the course of this study, he introduced the concept of "vitality" to explain original, or natural, health and, to give support to this concept, he performed a comparative investigation on health using smokers and nonsmokers.

When impaired vitality and neurosis are retarded as a disturbance of SD, SS becomes problematic. The framework of SS can be a cause of impaired vitality or neurosis in the sense that it suppresses SD. In connection with stress, it may be said that when a living body encounters stress, SD is suppressed within the framework of SS, leading to impaired vitality.

From this viewpoint, Chiba regarded smoking as a means of adapting oneself to SS and performed various investigations (CMI survey and a survey on smoking in both smoking and nonsmoking members of the Okayama City Junior Chamber of Commerce and Industry or their acquaintances) under the expectation that smokers might be more apt to show neurotic tendencies than nonsmokers.

In addition, 352 married couples gathered mainly through various friendship magazines, were subject in a study

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using the CMI survey form and the author's own survey form to test his hypothesis.

3. Directions for future studies

From the studies conducted so far, we can speculate that smoking functions to reduce certain kinds of mental stress, and that cessation of smoking leads to a transient increase in various kinds of stress. To proceed with this kind of study, it is now essential to examine all aspects of stress in human psychology so that the relationship between smoking and the cessation of smoking and stress can be better understood. We forecast that future studies in this field will attempt to clarify the relationship between mental stress (in the sense referred to above) and smoking/ex-smoking with comprehensive approaches covering not only physiological and pharmacological aspects but socialogical and psychological viewpoints as well.

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CONCLUSION

As stated above, the effects of smoking on health have been studied in a wide range of fields, and studies in each field are making steady progress. However, when seen from the total viewpoint of "medicine for smoking," which attempts to clarify the somatic and mental effects of smoking, the findings obtained so far are partial at best and many problems remain unsolved.

Consequently, in order to understand the various long-term effects of smoking on health, we should undertake further studies in the above-mentioned fields and at the same time cultivate new fields of study so that we may further our knowledge on the subject of smoking and health.

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2504066758

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